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CONTENTS

Abstracts of papers presented at annual meeting, supplementary to July number 805

The bacteriology of Swiss cheese. III. The relation of acidity of starters and of pH of the interior of Swiss cheese to quality of cheese. W. G. FRAZIER, W. F. JOHNSON, JR., J. R. BRADY, and G. J. RAMSTAD 809

Vitamin D studies in cattle. I. The antirachitic value of hay in the ration of dairy cattle. C. J. HUFFMAN and C. W. DUNCAN 831

Period of incubation and the direct titratable chloride value of milk. PAUL F. SHARP and EMMA B. SPITZER 837

The advantages of skim-milk agar for the determination of the sanitary quality of market milk. C. E. BARTRAM and C. K. SHARP 843

Vitamin A content of pasture plants. ELLA WOODS, F. W. ATKESON, HARRY WELLHOUSEN, and R. F. JOHNSON 847

Studies on the chemical composition of the blood of dairy cattle. A. H. VAN LANDINGHAM, H. O. HENDERSON, and G. A. BRAUCHER 857

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JOURNAL OF DAIRY SCIENCE

VOLUME XVIII AUGUST, 1935 NUMBER 8

ABSTRACTS OF PAPERS PRESENTED AT ANNUAL MEETING, SUPPLEMENTARY TO JULY NUMBER

E3. Farm Account Work Through D. H. I. A. Testing. A. J. CRAMER, Wisconsin College of Agriculture.

Wisconsin dairymen in Dairy Herd Improvement Association work have made farm account record keeping play a big part in building their farm income the past three years. No matter how good a dairy may be it cannot make up for the losses caused by poor crop production.

The farm account records call for—

1. Inventories of land, buildings, machinery, livestock, feeds, and miscellaneous supplies, taken at beginning and end of the year.
2. All farm expenses and receipts.
3. Production records of crops and livestock crop acre yields and the production of different classes of livestock.

Most testers spend 20–30 minutes each month with a member helping him to make entries in his farm account record book.

The problems which must be met are:

1. The records too often are not finished when sent in.
2. Some testers are not farm management minded.
3. Some inaccuracies in keeping records were found.
4. The new simplified book has encouraged many farmers to keep farm account records with their dairy herd records.

In 1932, there were 157 members completing books in 45 associations. Now there are 2,048 books being kept with the help of 81 testers. This is a 1200% increase over the first year the work was started.

Six of the most important factors on dairy farms of Wisconsin are:

1. Number of crop acres.
2. Production of butterfat per cow.
3. Crop yields per acre.
4. Per cent of crop land in alfalfa.
5. Livestock returns per $1.00 worth of feed used.
6. Diversity of farm income.

Wisconsin Farm Accounts Records were first kept in 1920 with one tester named Donald Mitchell in Waukesha County, and in 1922 with a second tester named Nander Nelson in Washington County, and in 1929

with a group of 5 testers all in Pierce County who kept records on 101 farms. Then in 1932 a special effort was made by our present Dean Christensen of the College of Agriculture who appointed a committee to consider the advisability of combining the functions of the two units of the Farm Management Department and the Dairy Records Department. The result was the office of Farm Accounts and Dairy Records. We are now starting the third year of record work with this combined office.

The first farm account record book which is discarded had a page-distribution of items by enterprises. This included inventories, purchases, sales, breeding records and management records, bills payable and receivable, all labor costs, feed crop costs, personal and household expenses, products supplied the family from the farm, etc. There were so many records that it confused the farmer and he did not wish to keep it .

The new book is put up in the form of a column distribution of receipts and expenses, with inventories and crop production records at one place in the book. We have left out half of the forms which were in the older book.

Concerning the office set-up, there are four specialists for the dairy work and two for the farm management work. There is one full-time clerk who works on the records while 5 other clerks help where work is to be done.

The record books are supplied to the cooperators (D. H. I. A. members) free of charge by the College of Agriculture; other people are charged 10 cents a copy.

The testers are given training along farm management lines each year through their short course training and through one or two 3-day schools. These schools familiarize the tester with the possible results to be obtained in keeping farm account records. Usually one day is spent going over the account book with the testers showing them how items are entered. Each tester, when hired, understands that his work consists of helping with the dairy records, and in keeping the complete record of farm income and expenses.

The tester knows that the success of his testing work hinges around increased income to the dairyman. Often the tester can help find and remedy the weak places in the farm business where records are available. The extension specialists spend time with the tester helping him with the farm account record work.

We have started most of the books after crops are harvested, after October 1 to April 1. The books are usually started at the beginning of the association year so they may be closed when the herd records are completed. We plan to get the books back to the farmers before spring work starts. They can think over the suggestions made and make changes in their cropping system for the next year.

The question often raised concerns the ability of the tester to carry on both lines of record work. I think our testers are doing as much D. H. I. A. record keeping as testers in other states with additional Farm Account records to be kept.

With the identification of all animals, the breeding and production records necessary to get daughter-dam comparisons, etc., this farm account work is the easiest work the tester is carrying. If testers are keeping the farm account records on the farm, the monthly feed records for the individual cows may be omitted, because we will get from the farm record book the feed efficiency figure which has been used more effectively than have the feed records obtained in connection with the dairy herd work alone. Usually the dairyman culls more on production than on feed efficiency of his cows.

We are looking for a better class of tester than we have in the past. Usually the tester who has kept up his herd records also does a good job with his farm account records.

The farm account record is voluntary on the part of the farmer. Some farmers prefer to keep their own records. Some farmers are requesting this work as time goes on so that the need for testers trained along farm management lines is increasing rather than decreasing.

E8. County feeding programs. H. R. SEARLES, Minnesota College of Agriculture.

Our extension program is being developed more and more around programs developed by groups of interested people in the counties with the assistance of the county agents and extension workers.

Because dairying is the most important farm enterprise in many of our Minnesota counties, there developed a desire on the part of a few counties to form a county dairy committee to study the needs of the county, and to formulate a county dairy program.

The committees as they have developed are made up of representatives of every active organization in the county, both farmers and business men. In most cases the members were elected or appointed by the organization that they represented.

This county committee of 30 to 40 men spend about two days with the county agent and extension dairyman in making up their program with a sub-committee in charge of each project.

The following projects are representative: Sires and Breeding, Testing, Marketing, Consumption, Disease Control, Crops and Feeding.

These committees give the county agent a working group to take over the leadership in developing a program on their project.

Several of the sub-committees have been very active and have added tremendously to the effectiveness of the county programs. They have

assisted in maintaining cow testing associations, in better bull placement and exchanges, in surveys of consumption and advertising campaigns for increasing consumption of dairy products; have held meetings and helped with the alfalfa and better pasture problems.

What place they will occupy in the future we do not know, but they should prove a real factor in developing a local dairy extension program.

E10. Record Keeping and Reporting in 4-H Dairy Club Work. R. G. CONNELLY, Virginia Polytechnic Institute.

Where judiciously administered, 4-H dairy club record keeping and reporting will provide an effective extension method for teaching rural boys and girls improved methods of dairy management.

Since 4-H dairy club work is a specialized educational enterprise for rural youth, a system of record keeping on the projects seems essential to the success of that enterprise. Such a system should provide technical dairy instruction; it should instill a scientific, as well as a business, attitude in rural youth towards the problems of dairying; it should provide for experience in intelligent dairy management; and finally, it should provide club members and others a basis for measuring project progress and improving dairy farm practices.

The purposes and applicability of 4-H dairy record keeping and reporting systems are manifold and extensive. They may serve in developing general extension programs in new communities; they have served as a mainstay in support of established extension programs, when the welfare of those programs was in the balance.

Any dairy record keeping and reporting system should not serve only as a basis for making 4-H club awards. It should be a means of developing purposeful dairy teaching matter on a simple plan, pertinent to the club members project. Furthermore, it should serve to improve dairy methods and the standards of rural living.

The records essential to the development of any 4-H dairy project should be classified to make record keeping and reporting easy. Names of animals, their breed, birth dates, pedigrees, etc., are semi-permanent records and should be reported once at the beginning of the project. Records concerned with yearly inventories on all animals, breeding dates, calving, feed consumption, growth, milk and butterfat production, and cost accounts change continuously, and therefore provision should be made in the state extension office for collecting and filing these records, since they should be basically important in developing the 4-H dairy teaching program.

Under proper cooperative conditions a 4-H dairy record keeping and reporting system may be adapted to fit the extension time and personnel in most any state and still yield worth while results. At present there are at least three general 4-H dairy record systems in effect. The first

and least involved is that in which the club member furnishes the essential data on himself and his club animal for entering a 4-H dairy project. He then submits to his county agent or club leader a yearly record on his project at the close of the project year. This system, though receiving rather limited supervision, has served to open the way for other more comprehensive record keeping systems.

A second system requires the club member to keep a complete yearly record book on his project which he submits along with a yearly achievement report to one of the local or state extension workers for a rating. Usually the local leader or county agricultural agent or club agent inspect the book during the progress of the project and make suggestions, but the responsibility for keeping the record up-to-date rests squarely on the club member.

A third, rather intensive system requires the club member to keep a yearly record book similar to the present Dairy Herd Improvement Association herd book. Monthly record reports are also required. At the end of the year the record books are collected, inspected and rated as a basis for making awards and planning the 4-H dairy extension program. In this system the relationship between the club member and extension service becomes more intimate than in the other systems, since closer supervision is necessary to make the system function. This system has yielded excellent results in several states.

If the purpose of 4-H dairy record keeping and reporting are to be served, the records must be carefully analyzed and used in the 4-H teaching program. The methods of analyzing and using club record information will depend upon the facts needed in the teaching program. Usually a simple summary of the records, with a computation of averages for comparative purposes, will be enough to direct the club member's attention to his project accomplishments.

Where monthly records are received they may be used effectively in developing 4-H dairy project interest or they may be sent in a monthly 4-H club letter to the club member. Honor Rolls supplemented with timely subject matter have proved particularly effective in at least one state.

In any event all the yearly 4-H records should be collected, inspected and officially acknowledged. Each club member should also receive from the state extension office a tactfully prepared letter with regard to his year's record work to give encouragement and at the same time to call his attention to points of improvement. Certificates of achievement might be issued as an acknowledgment of satisfactory project completion and the fact that the club member has qualified to proceed t othe next higher 4-H project, towards the ultimate fulfillment of the state 4-H dairy extension program.

E18–E22. Report of the Quality Improvement and Product Consumption Committee.

Committee members: A. J. Mann, Connecticut; C. A. Hutton, Tennessee; Fred Abbott, California; E. C. Scheidenhelm, Nebraska; A. C. Baltzer, Michigan, Chairman.

A questionnaire of 14 questions pertaining to (a) quality milk and cream production and (b) consumption of dairy products by producers and consumers was sent by the committee members to each state. Forty-two replies were returned. In general the dairy department of each state agricultural college was responsible for the replies received.

There was a unanimous opinion that the quality of both raw and finished dairy products has improved in tht past few years.

The changes effected in quality cream production include the following:

First, a gradual decrease in the number of cream stations; second, the cream stations now operating are tending to improve the sanitary conditions of the stations; third, a higher per cent of low acid cream is being received; fourth, cream sediment testing is increasing; fifth, the time of cream deliveries is shortening; sixth, refrigeration as an aid in quality production is growing.

Payment for cream by quality grade is practiced generally in the Pacific coast states and inclined to become a more general practice in the middle west. Very few if any cream stations are operating in the Pacific coast states.

The activities of the federal government during recent months resulting in the confiscation of butter containing extraneous matter has been the spur to improve the quality of dairy products in the majority of states. This has not burdened the producer with more expensive equipment according to the answers and in only a few instances has confiscation barred some producers from marketing products. The unanimous opinion of the states was that it has not had any unfavorable consumption reaction. The majority of the states report that some dairy products have been condemned but the percentage is less than 1 per cent.

A decided change is occurring in the states in regard to the standard of fluid milk offered for sale. It is improving in healthfulness; first, by reducing the incidence of cattle disease—removing T. B., Bang's and mastitis' reactors; and second, in quality by stepping up of the cooling requirements and sanitary conditions under which milk is being produced. The highest quality fluid milk requirements are found first, in the eastern section of the country; second, in the Pacific coast regions. Milk is not sold by quality grade, but largely by butterfat content, according to the report from these states.

Now to summarize the quality phase of this report, the committee wishes to express the opinion that, first, quality cream production increases

as the cream stations decrease in prominence. More frequent delivery, lower acidity cream, cream more free of sediment, cream with a more desirable butterfat content, and a slightly growing tendency for payment according to quality, are all factors tending to enhance quality cream production and diminish the importance of the cream station.

Second, the efforts of the department of pure food and drugs' drive to remove from consumption channels, dairy products containing extraneous matter, has served a good purpose. The amount of product confiscated is minor but the psychology of enforcement of existing laws has had a good effect.

Third, wholesome cooperation by the producer tending to improve the health standards of his herd is lending itself to a higher standard quality product.

Fourth, there has been a growing tendency for municipalities to, first, revise and bring up to date their milk ordinances, and second, for municipalities in the past without such ordinances, to make use of stronger milk ordinances and adopt in many cases the United States Public Health Milk Ordinance for the improvement of the local milk supply.

The questionnaires bring out, fifth, a decided need for enforcement of the laws pertaining both to milk and cream production. It is not the sense of the questionnaires that more laws are needed but that stricter enforcement will be helpful.

The second part of our report concerns itself with the problem of dairy product consumption. The substance of our questionnaire points out a decided lack of, first, information and second, any organized effort by agencies within the states to enlarge the consumer purchasing and producer consumption of milk and dairy products. The Pacific coast states again seem to lead the country in this regard. The dairy council activities in this region and similar activities along the Atlantic seaboard states are outstandng.

In making recommendations designed to get sustained and enlarged consumption of dairy products by consumers, the most commonly recommended means are advertising and education.

To summarize the results of this part of our report it is the sense of this committee that a fertile field exists to enlarge consumption of milk and dairy products, first, among producers themselves; second, that consumers will respond and enlarge the consumption of dairy products by emphasizing health values, safety, economy, and flavor and quality.

THE BACTERIOLOGY OF SWISS CHEESE

III. THE RELATION OF ACIDITY OF STARTERS AND OF pH OF THE INTERIOR OF SWISS CHEESES TO QUALITY OF CHEESES

W. C. FRAZIER,* W. T. JOHNSON, JR., F. R. EVANS, AND G. A. RAMSDELL
Research Laboratories, Bureau of Dairy Industry, Washington, D. C., and Grove City, Pa.

It has been shown in the preceding paper of this series[1] that *Streptococcus thermophilus* is active in carrying on a lactic acid fermentation during the first few hours that Swiss cheese is in the press and that *Lactobacillus helveticus* or a similar lactobacillus subsequently continues the fermentation. The pH values of the interior of the cheese have been used as indication of the progress of the fermentation and in particular of the effectiveness of the two kinds of starter bacteria, the lactic streptococci and the lactobacilli.

Most of the results included in the previous paper were obtained with small cheeses. It has long been realized that results with small cheeses do not always parallel results with full sized wheels. One reason is that the interior of the larger cheeses cools more slowly than the interior of smaller cheeses. The fairly common practice of cooling the kettle contents by the addition of cool water or whey just before the curd is dipped makes the temperature of the larger cheeses more like that of the smaller cheeses during the early hours of the cheese in the press. The cheeses studied in this paper were wheels with a green weight of about 155 to 160 pounds made under factory conditions at the Grove City Creamery, Grove City, Pa.

The milk used in making the Swiss cheese had for the most part a long methylene blue reduction time. The pH of the milk in the kettle was about 6.5 or 6.6 and the titratable acidity about 0.17 per cent. The fat content of the whole milk was from 4.3 to 4.9 per cent and after standardization the kettle milk contained from 3.5 to 3.8 per cent of fat. Most of the cheeses studied were not experimental but were made for the market.

The starters used were *Streptococcus thermophilus*, C_2 or C_3 strain, *Lactobacillus helveticus*, 39a strain, and *Propionibacterium Shermanii*, No. 62 strain.

* Resigned September 15, 1934.

Received for publication February 23, 1935.

[1] Frazier, W. C., Burkey, L. A., Boyer, A. J., and Sanders, G. P. The bacteriology of Swiss cheese. II. The bacteriology of Swiss cheese in the press. JOURNAL DAIRY SCIENCE 18: 373–388. 1935.

Methods of sampling and examination were as described in the preceding paper of this series.[1]

RESULTS

Since a pure culture starter contains only one kind of bacterium, the acidity of the starter after incubation at a given temperature will usually indicate the stage of growth of the bacteria and may indicate the likelihood of the culture being effective at the proper time in the cheese mass. A study has been made of the starters used in the Swiss cheeses at Grove City over a period of five years. Cheeses were not made continuously over that period, and cheeses included in special experiments were not included in the study.

Starters of *L. helveticus* (39a) were grown in sterilized skim milk at 37.5° to 39° C. for twelve hours. The higher incubation temperatures were used during a period when a more heat resistant organism was needed. The relation of the titratable acidity of the 39a starter used to the final grade of 862 cheeses made is shown in Table 1. It will be noted that of cheeses

TABLE 1

Acidity of 39a starters in milk and grades of cheeses made with them

ACIDITY OF 39a STARTER	NUMBER OF CHEESES	FANCY AND NO. 1	SPECIAL	NO. 2 AND NO. 3
per cent		*per cent*	*per cent*	*per cent*
< 0.90	40	62.50	12.50	25.00
0.90–0.94	20	85.00	5.00	10.00
0.95–0.99	43	53.49	32.56	13.95
1.00–1.04	114	67.54	21.93	10.53
1.05–1.09	115	63.48	26.96	10.43
1.10–1.14	121	54.55	32.23	13.22
1.15–1.19	190	56.84	24.21	18.95
1.20–1.29	91	39.56	30.77	29.67
1.30 and over	128	39.06	39.06	21.86
All of 1.2 and over	219	39.27	35.62	25.11
All of 1.0 to 1.09	229	65.07	24.45	10.48

made with starters of 1.0 per cent acidity or over, the highest percentage of Fancy and No. 1 cheeses was made with 39a starters of about 1.0 to 1.09 per cent acid; and that results with starters with 1.1 to 1.14 per cent acidity were almost as good, although the percentage of Fancy and No. 1 cheeses decreased some and the percentage of No. and No. 3 (grinder) cheeses increased. A starter acidity of 1.15 to 1.19 per cent gave a still greater percentage of No. 2 and No. 3 cheeses. When a starter of 1.2 per cent acidity

[1] Frazier, W. C., Burkey, L. A., Boyer, A. J., and Sanders, G. P. The bacteriology of Swiss cheese. II. The bacteriology of Swiss cheese in the press. JOURNAL DAIRY SCIENCE 18: 373–388. 1935.

or over was used, however, the quality of the cheeses was considerably poorer. There was a marked increase in percentage of No. 2 and No. 3 cheeses and a marked decrease in percentage of Fancy and No. 1 cheeses, as shown in Table 1. Of all cheeses made with starters of 1.2 per cent acidity and over, 39.27 per cent were Fancy or No. 1 cheeses and 25.11 per cent were No. 2 or No. 3 cheeses, while of all cheeses made with a starter of 1.0 to 1.09 acidity, 65.07 per cent were Fancy or No. 1 cheeses and only 10.48 per cent were No. 2 or No. 3 cheeses. The high percentage of cheeses of good quality made with starters of less than 0.9 per cent acidity as shown in Table 1 is misleading. The 40 cheeses were made in two different series. Twenty cheeses from one series graded 80 per cent Fancy and No. 1 cheeses and 20 per cent Special cheeses, but the other 20 cheeses in a different series graded 50 per cent Fancy and No. 1 cheeses, but 50 per cent No. 2 and No. 3 cheeses. In the first series the milk was good enough so that a weak 39a starter was satisfactory, but a similar starter in the second series produced cheeses half of which were of low quality.

The data lead to the conclusion that a 12-hour 39a starter in milk should have a titratable acidity of not over 1.19 per cent and not less than 1.0 per cent, preferably between 1.0 and 1.09 per cent. Experience has shown, however, that when there is difficulty in getting the 39a organism to grow properly in the cheese the use of a starter with a titratable acidity of 1.1 to 1.14 per cent is preferable.

In Tables 2 and 3 are shown results of studies of cheeses made with *Str. thermophilus* (C_2or C_3) starters of various acidities in milk and in whey, respectively. All cheeses in which the 39a culture did not develop properly

TABLE 2

Acidity of C_3 starters in milk and grades of cheeses made with them

ACIDITY OF C_3 STARTERS—MILK	NUMBER OF CHEESES	FANCY AND NO. 1	SPECIAL	NO. 2 AND NO. 3
per cent		*per cent*	*per cent*	*per cent*
Less than 0.75	17	52.94	29.41	17.65
0.75–0.80	112	67.86	22.32	9.82
Over 0.80	30	50.00	36.67	13.33

TABLE 3

Acidity of C_3 starters in whey and grades of cheeses made with them

ACIDITY OF C_3 STARTERS—WHEY	NUMBER OF CHEESES	FANCY, GOOD AND FAIR NO. 1	POOR NO.1	SPECIAL	NO. 2 AND NO. 3
per cent		*per cent*	*per cent*	*per cent*	*per cent*
Less than 0.30	39	33.33	23.08	38.46	5.13
0.30–0.33	112	41.07	22.32	33.93	2.68
0.34 and over	40	17.50	47.50	27.50	7.50

were eliminated from consideration. The data in Table 2 indicate that a titratable acidity of 0.7 to 0.75 per cent is desirable in a milk starter of *Str. thermophilus* and of 0.3 to 0.33 per cent in a whey starter.

As has been stated in a previous paper, the activity of *Str. thermophilus* during the first few hours of the cheese in the press is indicated by changes in pH during this period. The pH of the interior of the cheese three hours after dipping has been used to determine the activity of the organism. Too little or too much activity will be apt to harm the quality of the cheese. The rate of acid development in the early hours of the cheese in the press can be regulated by varying the quantity of active *Str. thermophilus* starter added to the kettle milk.

In Table 4 are shown the results of studies in which the pH of 353 cheeses was determined three hours after the curd was dipped. All cheeses

TABLE 4

The pH of the interior of the cheeses three hours after the curd was dipped and grades of the cured cheeses

pH OF CHEESES—3 HOURS	NUMBER OF CHEESES	FANCY AND NO. 1	SPECIAL	NO. 2	NO. 3—GRINDERS
		per cent	*per cent*	*per cent*	*per cent*
6.10–6.19	31	64.52	25.80	6.45	3.23
6.00–6.09	131	73.28	22.90	3.82	0.00
5.90–5.99	149	61.74	29.53	5.37	3.36
5.80–5.89	42	52.38	35.71	11.91	0.00

made with a weak 39a culture have been eliminated from consideration. It will be seen that the greatest percentage of cheeses of high grade and the lowest percentage of cheeses of low grade resulted when the interior of the cheeses had a pH value of from 6.0 to 6.09 after three hours in the press. There were no No. 3 (grinder) cheeses in this group. It is interesting to note that likewise there were no No. 3 cheeses but a high percentage of No. 2 cheeses in the group with a pH of 5.8 to 5.89 after three hours. These low pH values indicate great activity by *Str. thermophilus*. Such cheeses drain very rapidly and have comparatively little growth of *L. helveticus* later in the cheese. The cheeses usually rise high in the warm cellar and are overset, but they do not ordinarily have the defects which would put them into the grinder class.

In Table 5 are shown pH readings of samples from the interior of the cheeses eight hours after the curd has been dipped into the hoop in the press. The results indicate that a pH of 5.5 to 5.69 gave the smallest percentage of low grade cheeses, but that pH 5.7 to 5.89 was practically as satisfactory.

The pH of the interior of the cheese 21 hours after dipping is taken as an indication of the growth and activity of the lactobacillus starter (39a).

TABLE 5

The pH of the interior of the cheeses 8 hours after the curd was dipped and grades of the cured cheeses

pH OF CHEESES—8 HOURS	NUMBER OF CHEESES	FANCY AND NO. 1	SPECIAL	NO. 2 AND NO. 3
		per cent	*per cent*	*per cent*
5.30–5.49	32	28.13	34.37	37.50
5.50–5.69	30	50.00	33.33	16.67
5.70–5.89	50	56.00	24.00	20.00
5.90–6.09	38	47.37	36.31	26.31
6.10 and over	28	14.29	60.71	25.00

Table 6 shows the relation of the 21-hour pH to the final grades of the cheeses. It will be noted that the best grades of cheese were obtained when the pH values at 21 hours were 5.0 to 5.09, but that almost as good a cheese was obtained when the pH value at 21 hours was 5.1 to 5.14. When the pH is 5.2 or over the grade of the cheeses is very likely to be poor. The summary in Figure 1 of results from all cheeses with a pH of 5.2 at the end of 21 hours and over and from cheeses with less than 5.2 emphasizes the danger of a pH value of 5.2 or more after 21 hours in the press, wherein only 30.39 per cent were Fancy or No. 1 cheeses and 43.81 per cent were No. 2 or No. 3 cheeses.

A reason was sought for a grade of Fair No. 1 or better of 50 (of 249) cheeses in spite of a pH of 5.2 or over after 21 hours on the press. As is shown in Table 7 these cheeses were more alkaline during the early hours in the press than most cheeses, that is, *Str. thermophilus* was less active than usual. It will be noted that the better the grade the more alkaline was the average pH after three hours and the more acid the pH after 21 hours.

TABLE 6

The pH of the interior of the cheeses 21 hours after the curd was dipped and grades of the cured cheeses

pH OF CHEESES—21 HOURS	NUMBER OF CHEESES	FANCY AND NO. 1	SPECIAL	NO. 2 AND NO. 3
		per cent	*per cent*	*per cent*
< 5.0	8	62.50	25.00	12.50
5.00–5.04	133	65.41	24.81	9.77
5.05–5.09	235	63.40	25.11	11.06
5.10–5.14	135	58.52	31.85	9.63
5.15–5.19	100	55.00	31.00	14.00
5.20–5.29	79	46.84	27.85	25.31
5.30–5.39	61	18.03	24.59	57.38
5.40–5.49	42	21.43	21.43	57.14
5.50 and over	12	25.00	25.00	50.00
All 5.2 and over	194	30.93	25.26	43.81
All < 5.2	623	61.32	27.45	11.23

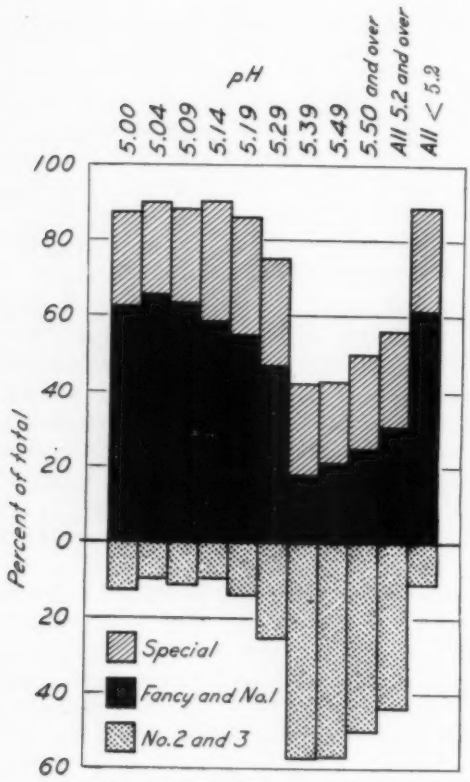

FIG. 1. Relation of pH at 21 hours to grade of cheese.

All of these cheeses showed a slow increase in acidity during the early hours in the press, but the increase was steady and undoubtedly continued after the 21-hour period. These results agree with the belief of experienced cheesemakers that the less starter it is found necessary to use, the more likely are good cheeses to result. The good results obtained with 39a

TABLE 7

Changes in pH of all cheeses of good grade with 21-hour pH of over 5.2

Grade	3 HOURS		8 HOURS		10 HOURS		21 HOURS	
	No. of cheeses	Average pH	No. of cheeses	Average pH	No. of cheeses	Average pH	No. of cheeses	Average pH
Fancy	5	6.21	.....	.....	4	5.53	5	5.28
Good No. 1	20	6.10	12	5.85	3	5.66	25	5.32
Fair No. 1	25	6.08	16	5.79	4	5.64	50	5.35

starters with less than 0.9 per cent acidity in certain cases, as discussed above, were in cheeses in which there was slow development of acidity during the first hours in the press. In the presence of active gas-forming bacteria, however, slow working *Str. thermophilus* or *L. helveticus* starters or small amounts of them could not be used successfully.

DISCUSSION

Since it is usually possible to control to a great extent the rate of the lactic fermentation in Swiss cheese in the press by controlling the starters used in making the cheese, the cheesemaker should know first how to prepare the starters which will be most active in the cheese and, second, how much of these active starters should be used to cause the desired rate of lactic fermentation in the cheese in the press. The results of the above data would indicate that a 39a starter should have an acidity of about 1.05 to 1.09 per cent and the C_3 whey starter an acidity of 0.3 to 0.33 per cent. Enough C_3 starter should be added to cause the pH of the cheese to drop to about 6.0 to 6.09 in the first three hours of the cheese in the press and enough 39a starter should be added to give a pH of less than 5.2, preferably less than 5.15, after the cheese has been in the press for 21 hours. No changes can be made to influence the cheese on which the tests are run but the changes can be effected the next day. In practice it is not always possible to obtain the ideal pH value in the cheese, especially if the milk is defective. Some sources of milks are so infected with gas organisms that a large amount of *Str. thermophilus* starter is necessary to prevent bloating of the cheese in the press. The resulting cheese will probably not be of good quality but the "pressler" will have been avoided. Some milks are inhibitive to the lactobacillus starter and difficulty is experienced in obtaining a low enough pH value in the 21-hour old cheese. This trouble was encountered at the Grove City Creamery and it was found necessary to use a 39a starter grown at 38–39° C. with an acidity of about 1.1 to 1.15 per cent. And, with an exceptionally good milk, as has been stated above, a slow acting *Str. thermophilus* as well as a slow acting lactobacillus can be used with good results.

Of course the first requisite for the manufacture of Swiss cheese of high quality is satisfactory milk. And with good milk, correct manufacturing procedures and properly prepared and used starters, it should be possible to make Swiss cheese of high average quality.

SUMMARY

A study of a large number of wheels of Swiss cheese manufactured at a plant under factory conditions, in an effort to determine what acidity of pure culture starters and what rate of lactic acid fermentation in the cheese in the press were most desirable, led to the following conclusions:

1. With the milk used, a *Lactobacillus helveticus* milk starter with an acidity of 1.0 to 1.09 was most effective. This culture was grown at 37.5–39° C. for 12 hours.

2. A 12-hour, 37° C. milk starter of *Str. thermophilus* should have an acidity of about 0.70 to 0.75 per cent; and a whey starter should have an acidity of 0.30 to 0.33 per cent.

3. The pH of the cheese in the press, which is indicative of the activity of the starters, should be about 6.0 to 6.09 after three hours in cheese made from milk with a pH of 6.5–6.6.

4. The pH of the cheese in the press should be less than 5.2 after 21 hours and preferably should be between 5.15 and 5.0.

5. With good milk a slow development of acidity throughout the first 21 hours usually makes a good cheese. The pH of such cheeses may be as high as 6.1 to 6.25 after three hours in the press, and over 5.2 after 21 hours.

6. With a supply of good milk and with good manufacturing methods, the proper preparation and use of pure culture starters will give Swiss cheese of good average quality.

VITAMIN D STUDIES IN CATTLE

I. THE ANTIRACHITIC VALUE OF HAY IN THE RATION OF DAIRY CATTLE*

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For the past 12 years the calcium and phosphorus requirements of dairy cattle have been under investigation at this Station. During this period several long-time mineral feeding trials and many balance experiments have been carried out (1–4). In order to complete certain phases of this investigation, it became necessary to study the vitamin D requirement of dairy cattle and also the antirachitic value of common dairy feeds. These factors are essential for the proper utilization of calcium and phosphorus.

Extensive reviews of the literature bearing on the etiology of rickets and the effects of various curative agents have been made by Park (5), Hess (6), Blunt and Cowan (7), Sherman and Smith (8), and Browning (9). Consequently, only the literature pertaining directly to rickets in cattle will be given.

Rickets have been observed in calves by several early writers. In 1901 Law (10) listed it as a disease affecting cattle. In their 1914 edition, Hutyra and Marek (11) have a splendid photograph of a rachitic calf. In 1920 Becker (12) observed rickets in calves fed whole milk and grain. The California Station (13) reported that rachitic calves were common in the range herds of King County as early as 1922.

Rachitic calves were produced experimentally at this Station (14) as early as 1926 in connection with the heavy feeding of concentrates without the proper quality of roughage. Bechdel and coworkers (15) presented data in 1928 which indicated that vitamin D was beneficial to the bovine. Olson (29) reported in 1929 that rickets occurred among calves allowed free choice of feeds. The rachitic symptoms were associated with low hay intakes. In 1930 Huffman and coworkers (16) reported a case of rickets in a young animal which was fed a ration containing a limited amount of wheat straw. Hill (17) reported in 1930 that calves fed a rickets-producing ration and exposed to ultraviolet rays showed improved calcification of the bones. Rupel, Bohstedt and Hart (18) demonstrated in 1931 that rachitic

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calves responded promptly to the curative treatment of cod liver oil or sunshine. The effects of supplementing a rachitogenic ration with cod liver oil, sun-cured hay or sunshine were reported in 1931 (19). In 1932 Gullickson and Eckles (20) observed rickets in calves under farm conditions. In 1933 both the Pennsylvania Station (21) and the Wisconsin Station (22) reported in detail upon the vitamin D studies at their respective institutions. The characteristic symptoms of rickets in calves were described in the last two reports.

Hess and Weinstock (23) reported that green plants grown in the dark were devoid of antirachitic properties but such plants were rendered antirachitic by irradiation with ultraviolet rays. According to Steenbock and coworkers (24) the antirachitic potency of hay can also be increased by exposure to sunshine or to ultraviolet irradiation. Similar results have been reported by other investigators (25–27).

The purpose of this investigation is to help elucidate the vitamin D requirements of calves and also to study the antirachitic potency of hay.

EXPERIMENTAL

Twelve calves were used in the study of the antirachitic properties of hay. The calves were all healthy grade Holsteins except C–252 which was a Brown Swiss. From birth to 30 days of age the calves were fed whole milk twice daily in amounts according to the needs of each calf, after which time they were changed to a ration of skim milk and a mixture of corn and oats. At 90 days of age the corn and oats were replaced by the following basal rachitic grain mixture:

Yellow corn meal	50.0 per cent	Linseed oil meal	8.5 per cent
Ground oats	20.0 " "	Calcium carbonate	1.0 " "
Corn gluten meal	20.0 " "	Sodium chloride	0.5 " "

Monthly analayses of rachitogenic ration were made and showed the following variations:

Calcium	0.412 to 0.592	0.500 average per cent
Phosphorus	0.301 to 0.376	0.351 " " "
Magnesium	0.164 to 0.216	0.195 " " "
Nitrogen	2.92 to 3.41	3.10 " " "
Moisture	10.27 to 12.95	11.27 " " "

At 120 days of age the skim milk was discontinued. Five calves, C–98, C–100, C–109, C–148 and C–131 received sun-cured hay as an antirachitic supplement. Four calves, C–127, C–130, C–133, and C–165 received timothy hay which had been cured in the dark as a supplement. Two calves, C–216 and C–252, were fed sun-cured alfalfa hay as an antirachitic supplement. Calf C–144 was fed timothy ash as a supplement.

The sun-cured timothy hay was U. S. grade No. 2. The timothy which was cured in the dark was cut after sundown and placed upon racks in a dark room to cure. This hay was classed as U. S. grade No. 2. The sun-cured alfalfa which was U. S. Grade No. 1 was purchased from farmers within the vicinity of the Station. The vitamin D content of these hays was not determined by rat assays.

The calves were turned into a bare lot at night for exercise except during inclement weather. They were offered water twice daily. Shavings were used for bedding.

The animals were weighed every 10 days and measured for height at withers once a month.

Blood samples were obtained every 2 weeks, in many cases each week, from all of the experimental animals from the time they were placed on experiment until they were removed from experiment. The methods for the determination of plasma calcium and inorganic phosphorus have been previously reported (3).

The right 8th rib and in a few cases the right dental pad and a section of the orbital plate of each calf were cleaned, air-dried, sawed into desirable lengths, extracted with hot alcohol for 72 hours and finally dried in an oven. The samples were weighed into crucibles and ashed in a muffle for 12 to 15 hours. After cooling in a desiccator they were reweighed. The ashed samples were ground to pass a 20 mesh sieve. Aliquot samples were then taken for the determination of calcium, phosphorus and magnesium.

The methods for the collection of the excreta and of the analysis were the same as in previous work (28).

RESULTS

The results for representative calves are presented in Tables 1–7 inclusive.

Calf C–144. Timothy ash equivalent to 2 pounds of timothy hay per day was added to the ration of C–144 at 90 days of age to compensate for the calcium, phosphorus and magnesium contained in the hay. The plasma calcium and inorganic phosphorus had been normal up to this time. By 105 days of age the inorganic phosphorus had dropped to 5.61 mg. per 100 cc. of plasma. The symptoms of rickets were marked at 145 days of age although the plasma inorganic phosphorus had been low for some time. Table 1 shows a typical case of low blood inorganic phosphorus rickets. Several calves not included in this discussion, which had received only the basal rachitic ration, also became extremely rachitic.

Effect of Supplementing the Basal Ration with Sun-cured Timothy Hay

In order to determine the antirachitic value of sun-cured hay, 5 calves were fed the basal rachitic ration supplemented with sun-cured timothy.

TABLE 1

C–144. Effect of supplementing the rachitogenic ration with timothy ash

DATE	AGE	WT.	PLASMA		DATE	AGE	WT.	PLASMA	
			Ca	P				Ca	P
	days	*lbs.*	*mg. per 100 cc.*			*days*	*lbs.*	*mg. per 100 cc.*	
10–12–31	40	94	12.6	7.49	12–31–31	120	159	10.5	5.51
10–22	50	100	11.3	6.25	1–10–32	130	168	.....	.....
11–1	60	111	.....	.....	1–20	140	182	10.9	3.95
11–11	70	115	11.0	8.33	1–30[2]	150	169	10.2	4.46
11–21	80	119	10.2	8.01	2–9	160	185	.....	.....
12–1[1]	90	124	.....	.....	2–19	170	187	10.4	4.43
12–11	100	131	11.2	5.61	2–29	180	194	.....	5.53
12–21	110	140	.....	.....	3–10	190	200	9.6	4.11

[1] Timothy ash added.

[2] Legs bowed.

Calf C–98. This calf received alfalfa hay and 10 cc. of cod liver oil per day to 90 days and was then changed to the basal ration. The calcium and inorganic phosphorus values were 12.0 and 5.25 mg. per 100 cc. of plasma respectively at 150 days of age, as indicated in Table 2. The phosphorus value was slightly below the normal range for a calf of that age. At 193 days of age the plasma calcium and inorganic phosphorus values were 7.0 and 6.95 mg. respectively and by 210 days of age anorexia was exhibited. Stiffness was not observed until about 225 days of age. This animal suffered from low plasma calcium, normal phosphorus rickets at this time. It is of interest to note that tetany was not observed although the plasma calcium values were below 6.0 mg.

Sun-cured timothy hay was added to the ration at 254 days of age when the calf was suffering severely with rickets. The plasma calcium immediately increased to 11.3 mg. but the inorganic phophorus remained unchanged. It was planned to feed 3 pounds of timothy per day but due to anorexia, the calf would not consume this amount of hay until about 30 days from the time it was added to the ration.

During the period that rickets had developed the calcium and phosphorus intakes were approximately 14.5 and 9.1 gm. per day. After the sun-cured timothy had alleviated the anorexic and rachitic conditions the above intakes were increased to 20.0 and 11.3 gm. respectively. The addition of the hay also changed the calcium to phosphorus ratio from 1.6 : 1 to 1.8 : 1.

After the sun-cured timothy had been added to the ration, all of the plasma calcium values and most of the inorganic phospohrus values were normal until this animal was 540 days of age, but at this age the inorganic phosphorus values began to decrease. C–98 started to bloat at 543 days of age and anorexia was again manifested. The grain consumption began

TABLE 2

C–98. Effect of supplementing the rachitogenic ration with sun-cured timothy hay

DATE	AGE	WT.	PLASMA		DATE	AGE	WT.	PLASMA	
			Ca	P				Ca	P
	days	*lbs.*	*mg. per 100 cc.*			*days*	*lbs.*	*mg. per 100 cc.*	
12–19–30	150	276	12.0	5.25	5–12	660	636	13.4	5.39
1–19–31	180	310	.....	.....	6–11	690	640	14.2	5.49
2–17	210	305	6.5	8.65	7–11	720	708	14.5	4.92
3–19[1]	240	299	5.8	7.47	8–10	750	757	13.7	5.91
4–2[2]	254	301	7.0	5.95	9–9	780	796	12.6	5.40
4–18	270	297	11.3	5.87	10–9[4]	810	839	12.3	5.32
5–18	300	325	11.3	6.14	11–8	840	887	11.3	5.90
6–17	330	351	11.9	6.76	12–8–32	870	926	11.9	6.23
7–17	360	391	11.5	5.82	1–7–33	900	947	12.0	6.80
8–16	390	432	12.4	6.24	2–6	930	997	10.8	6.33
9–15	420	465	11.4	7.13	3–8	960	1054	11.0	5.52
10–15	450	487	11.7	6.57	4–7	990	1087	12.1	5.71
11–14	480	536	11.3	5.95	5–7	1020	1120	11.3	6.06
12–14	510	573	11.3	6.21	6–6	1050	1147	11.0	5.59
1–13–32	540	574	11.4	5.89	6–30[5]	1074	967	11.9	3.71
2–12	570	590	10.8	5.16	7–6	1080	973	10.0	4.19
2–29[3]	587	588	.....	6.04	8–5	1110	990	9.8	4.24
3–13	600	576	.....	6.04	9–5	1140	.....	11.2	5.38
4–12	630	608	12.5	5.18	9–29	1164	990	11.9	6.62

[1] Marked rickets.
[2] 3 lbs. sun-cured hay added per day.
[3] Hay increased to 4 lbs. per day.
[4] Conceived.
[5] Calved.

to decrease but the 3 pounds of hay were readily consumed. Stiffness was observed at 585 days of age or 42 days following the initial bloating and development of anorexia. The plasma inorganic phosphorus was slightly below normal during this period but the calcium was normal. The hay intake was increased at 587 days of age but it required about 30 days to get it up to 6 pounds per day. The appetite for grain increased at a slower rate as four months were required to get the consumption up to the level just prior to the last siege of anorexia and attack of rickets. As the appetite for grain improved, the appetite for timothy decreased, which, together with good gains in body weight, were probably responsible for the inorganic phosphorus values remaining slightly below normal.

C–98 manifested estrum and was bred at 555 days of age but conception did not occur. She did conceive at the second service at 814 days of age. The calf, which was carried 261 days and was dead at birth, weighed 82 pounds. Post-mortem examination did not reveal any abnormalities. All of the bones of the calf were normal and well calcified. The placenta was discharged within the normal time. This animal had suffered from rickets on two different occasions yet she did not experience difficulty in calving. She was kept for 88 days following parturition.

The data for the above period showing milk production, calcium, phosphorus and magnesium intakes, the grams of food phosphorus per pound of milk above maintenance, plasma calcium and inorganic phosphorus appear below:

PERIOD	WEIGHT	HAY	MILK	AVER. DAILY INTAKE			P INTAKE[2]	PLASMA	
				Ca	P	Mg		Ca	P
days	*lbs.*[1]	*lbs.*	*lbs.*	*gm.*	*gm.*	*gm.*	*gm.*	*mg. per 100 cc.*	
31	967	6	23.4	35.3	20.1	13.8	0.446	10.9	3.95
31	990	6	30.8	52.5	26.3	20.3	0.532	9.8	4.24
26	990[3]	6	27.6	66.5	35.0	25.8	0.909	11.5	6.00

[1] Weight at beginning of period.

[2] Per pound of milk above maintenance. Maintenance requirement is 1 gram of phosphorus per 100 pounds of body weight.

[3] Weight at end of last period.

During the first and second months when the phosphorus intakes per pound of milk above maintenance were 0.446 and 0.532 gm., the plasma inorganic phosphorus values were low for a heifer of this age. During the third month the consumption of grain had increased to 15 pounds per day and consequently the phosphorus intake increased to 0.909 gm. per pound of milk above maintenance which caused the plasma inorganic phosphorus to return to normal. There was a pronouced improvement in the general

condition of C–98 during the time she was milking. The stiffened and enlarged joints, which were so pronounced at the time of calving, had almost disappeared by the time she was slaughtered.

It is of importance to call attention to the fact that 6 pounds of sun-cured timothy hay was the only source of vitamin D and that this amount of hay provided sufficient vitamin D for maintenance, some growth and the production of 23.4 to 30.8 pounds of milk per day. Apparently, the improved calcium, phosphorus and magnesium intakes were important factors in helping to restore the plasma inorganic phosphorus to normal. The metabolism data for calcium, phosphorus, magnesium and nitrogen following the recovery from the first attack of rickets are shown in Table 3. The

TABLE 3

Metabolism data of C–98 and C–100 fed the rachitogenic ration supplemented with sun-cured timothy hay

CALF NO.		AVER. DAILY INTAKE	AVER. DAILY OUTGO			BALANCE	% STORED	PLASMA	
			Urine	Feces	Total			Ca	P
		gm.	*gm.*	*gm.*	*gm.*	*gm.*		*mg. per 100 cc.*	
C– 98[1]	Ca	21.77	0.36	10.40	10.76	11.01	50.6	11.3	6.56
	P	12.95	3.46	3.88	7.34	5.61	43.3		
	Mg	7.87	2.00	2.99	4.99	2.88	36.6		
	N	94.73	49.17	21.75	70.92	23.81	25.1		
C–100[2]	Ca	19.45	0.02	12.28	12.30	7.15	36.8	11.2	6.65
	P	12.04	2.94	5.38	8.32	3.72	30.9		
	Mg	7.15	1.94	2.83	4.77	2.38	33.2		
	N	90.46	54.02	24.09	78.11	12.35	13.6		

[1] 3 lbs. of hay per day. Age 363 days. Weight 417 lbs.
[2] 2 lbs. of hay per day. Age 296 days. Weight 410 lbs.

metabolism period lasted one week but no feed changes were made for several weeks prior to the collection period. The ash values for the moisture-free, fat-free 8th right ribs of C-98 and her calf are shown in Table 7.

Calf C–100. This calf consumed 15.4 pounds of sun-cured alfalfa hay in addition to the basal ration during the first 60 days of age. At this time the alfalfa was replaced by 2 pounds of sun-cured timothy hay per day. The body weights, plasma calcium and inorganic phosphorus values are shown in Table 4.

Two pounds of sun-cured timothy hay furnished a sufficient amount of the antirachitic factor to maintain a normal concentration of calcium and inorganic phosphorus in the plasma to about one year of age. The inorganic phosphorus values were slightly subnormal after this time but anorexia was not observed until 504 days of age and stiffness was observed 4 days later. The phosphorus intake during this period ranged from 10.6 to 15.2 gm. per day. The hay was increased to 4 pounds per day on the 519th day. Slight improvement in the rachitic condition was first observed

TABLE 4

Effect of supplementing the rachitogenic ration with sun-cured timothy hay

DATE	AGE	WT.	PLASMA		DATE	AGE	WT.	PLASMA	
			Ca	P				Ca	P
	days	*lbs.*	*mg. per 100 cc.*			*days*	*lbs.*	*mg. per 100 cc.*	
				Calf C–100					
1–25–31	120	223	11.2	6.54	3–20–32	540	589	11.5	4.18
2–23	150	243	11.2	7.81	4–19	570	620	12.4	5.08
3–26	180	257	10.7	8.20	5–18	600	618	12.1	5.27
4–25	210	293	10.6	7.82	6–18	630	670	13.7	6.35
5–25	240	327	10.5	7.04	7–18	660	676	14.2	6.04
6–24	270	376	10.7	6.70	8–17[2]	690	732	13.7	5.67
7–24	300	415	10.8	6.53	9–16	720	740	12.7	5.53
8–23	330	431	11.0	5.77	10–16	750	745	11.4	5.46
9–22	360	469	10.8	6.65	11–15	780	754	10.8	5.62
10–22	390	497	11.9	6.12	12–15–32	810	765	11.6	5.49
11–21	420	506	10.8	5.60	1–14–33	840	779	11.8	6.63
12–21–31	450	533	11.1	7.09	2–13	870	808	11.5	6.06
1–20–32	480	543	11.3	6.41	3–15	900	855	11.4	6.29
2–19[1]	510	575	10.6	4.75	4–7[3]	923	846	13.0	5.81
				Calf C–148					
3–21–32	20	98	12.5	5.61	7–19	140	165	.....	.....
3–31[4]	30	110	11.9	4.46	7–29[7]	150	165	12.0	3.12
4–10	40	118	11.3	5.84	8–8	160	167	10.4	2.58
4–20	50	129	11.4	6.22	8–18	170	172	9.7	3.93
4–30	60	144	10.1	5.43	8–28	180	170	.....	.....
5–10	70	154	.....	.....	9–7	190	171	9.9	2.96
5–20	80	166	10.0	5.04	9–17	200	175	9.6	3.23
5–30[5]	90	171	11.7	5.32	9–27	210	180	.....	.....
6–9	100	171	11.8	4.09	10–7	220	190	12.3	5.00
6–19	110	169	.....	.....	10–17	230	187	8.5	4.05
6–29[6]	120	168	12.2	3.95	10–19–32	232	.....	7.3	4.13
7–9	130	165	11.8	2.76					

[1] Anorexia and stiffness, sun-cured timothy increased to 4 lbs. per day.

[2] Hay increased to 6 lbs. per day.

[3] Slaughtered.

[4] Sun-cured timothy hay added to ration.

[5] Rickets.

[6] Added MgO to ration.

[7] Added bone-meal to ration.

at 543 days of age but this condition was probably due to lack of growth. The plasma calcium was normal but the inorganic phosphorus was subnormal. The improved condition only lasted about 30 days when the stiffness became more pronounced. At 681 days of age the timothy was increased to 6 pounds per day. There was another period of gradual improvement in the rachitic condition but anorexia was the predominant factor. The calcium and phosphorus intakes at this time were 25 and 15 gm. respectively. By 850 days of age anorexia had disappeared and the grain consumption had increased to such an extent that C–100 gained in weight and in height at withers and at 909 days she weighed 861 pounds. The stiffened and enlarged joints had almost disappeared but the inorganic phosphorus values remained below 6.5 mg. in most cases. C–100 was slaughtered at 923 days of age because of a foreign body. She had had 13 services but conception had not occurred.

The metabolism values for calcium, phosphorus, magnesium and nitrogen just prior to the development of rickets are shown in Table 3. The collection period lasted for one week but no feed changes were made for many weeks previous to this time. The ash values are shown in Table 7.

Calf C–109. Blood samples were not taken from this calf until 84 days of age but the calcium and inorganic phosphorus values at that time were 7.6 and 7.40 mg. per 100 cc. of plasma. One pound of sun-cured timothy hay was added to the basal rachitic ration at 87 days of age. The plasma calcium values remained below the normal range until the calf was slaughtered at 188 days of age. The inorganic phosphorus values were normal until 160 days of age but from then on they were below normal. Tetany was not observed at any time and the calf did not appear irritable. The knee joints began to enlarge but stiffness had not been observed. Postmortem examination revealed the usual rickety bones.

Calf C–148. Table 4 summarizes the data secured from C–148. This calf had low inorganic phosphorus values when it was bled at 20 and 30 days of age but the calcium values were normal. Sun-cured timothy hay was added to the ration at 30 days of age. The calf was offered more but would not consume more than one pound per day up to 90 days of age. Evidence of rickets was observed at this time when the calcium was 11.7 and the inorganic phosphorus was 5.32 mg. per 100 cc. of plasma. The rachitic condition became progressively worse and at 121 days of age, 10 gm. of magnesium oxide were added to the ration in an attempt to relieve this condition. There was an immediate increase in the consumption of hay and grain but the inorganic phosphorus continued to decrease. The body weight remained constant and the rachitic symptoms remained unchanged even after the hay and grain intakes were doubled. Fifty grams of bonemeal per day were added to the ration at 153 days of age but the increased calcium and phosphorus intakes did not prevent the calf from becoming

extremely rachitic. The condition was such that the animal could not walk without assistance. All other feeds were replaced with 20 pounds of whole milk at 211 days of age. The plasma calcium, inorganic phosphorus and magnesium values at 220 days of age were 12.3, 5.00 and 2.12 mg. per 100 cc., respectively. At 232 days of age this calf had tetanic convulsions, during which time the calcium, inorganic phosphorus and magnesium values were 7.3, 4.13 and 0.99 mg., respectively. This calf presented a typical case of low-calcium, low-phosphorus rickets. The cause of the tetany may have been due either to the low concentration of calcium in the plasma, the low concentration of magnesium, or the subnormal concentrations of both of these elements.

Calf C–131. The concentrations of calcium and inorganic phosphorus in the plasma of this calf were normal during the first 46 days of age. At 65 days of age the plasma calcium dropped to 7.6 mg. per 100 cc. but the inorganic phosphorus remained within the normal range. Sun-cured timothy hay was added to the ration at 93 days of age. This was sufficient to maintain the normal concentration of inorganic phosphorus until 136 days of age. The first evidence of rickets was observed at 178 days when both the plasma calcium and inorganic phosphorus were much below normal.

Considerable difficulty was encountered in getting this calf to consume enough nutrients either as hay or grain. Originally it had been planned to feed the calf 2 pounds of sun-cured timothy but due to anorexia, the amount of hay consumed was usually about 1 pound per day.

At 239 days of age C–131 became extremely rachitic and was only able to get up with considerable difficulty. The calf was changed to another experiment at this time.

Effect of Feeding Timothy Hay Cured in the Dark

Calf C–127. The first indications of rickets were observed in this calf at 84 days of age when the calcium was 7.8 mg. per 100 cc. of plasma but the inorganic phosphorus was normal. One week later tetany was manifested so the ration was supplemented with timothy hay which had been cured in the dark. The plasma calcium and inorganic phosphorus values at this time were 6.4 and 7.53 mg., respectively. Tetany was observed at 94 and 95 days of age. Difficulty was encountered in getting this animal to consume hay and at no time did it consume more than 1.5 pounds per day. By 165 days of age the hay consumption had decreased to less than 1 pound per day. It was impossible to determine the antirachitic value of this hay because of the small amount consumed. However, the feeding of sun-cured timothy from the same field and at the same level was also ineffective in curing or preventing rickets (Calf C–109). The plasma calcium and inor-

ganic phosphorus values were all markedly subnormal during the last 3 months of life.

Calf C–130. The plasma calcium and inorganic phosphorus values were normal until 76 days of age at which time the calcium was 8.6 mg. per 100 cc. The inorganic phosphorus remained normal until 133 days of age. Timothy hay which had been cured in the dark was added at 99 days of age. Difficulty was encountered in getting C–130 to consume hay although about 2 pounds per day were consumed for 2 months. This amount of hay would not have been sufficient to cure rickets even though it had been sun-cured. The calf was removed from this phase of the experiment at 269 days of age.

Calf C–133. The plasma calcium and inorganic phosphorus of this calf was also normal until 73 days of age when the calcium dropped to 9.2 mg. per 100 cc. The inorganic phosphorus remained within the normal range until 126 days of age. Timothy hay which had been cured in the dark was added to the ration at 92 days of age but the consumption did not exceed 1.5 pounds per day at any time. The calf was slaughtered at 170 days of age at which time the plasma calcium and inorganic phosphorus values were 8.5 and 4.51 mg. per 100 cc.

Calf C–165. The timothy hay which was cured in the dark was added to the ration of this calf at 40 days of age while the plasma calcium and inorganic phosphorus values were still normal but the calf would not eat enough hay to prevent the onset of rickets. The calf began to manifest anorexia even though the plasma calcium and inorganic phosphorus values were still normal. The average daily consumption during the 152 days of hay feeding was only 0.5 pound. It is of interest to note that the blood plasma indicated the simultaneous development of the low-calcium, low-phosphorus type of rickets, as shown in Table 5.

TABLE 5

C–165. Effect of supplementing the rachitogenic ration with timothy hay cured in the dark

DATE	AGE	WT.	PLASMA		DATE	AGE	WT.	PLASMA	
			Ca	P				Ca	P
	days	*lbs.*	*mg. per 100 cc.*			*days*	*lbs.*	*mg. per 100 cc.*	
5–5–32	20	101	13.1	6.44	8–13	120	191	.....	.....
5–15	30	110	12.4	6.51	8–23	130	194	8.7	4.30
5–25[1]	40	123	11.9	6.28	9–2	140	189	8.9	4.63
6–4	50	134	.....	.....	9–12	150	192	.....	.....
6–14	60	145	10.7	7.35	9–22	160	196	8.5	4.41
6–24	70	150	10.1	7.81	10–2	170	199	10.5	4.96
7–4	80	158	.....	.....	10–12	180	196	6.6	3.62
7–14	90	164	10.7	8.68	10–22	190	201	.....	.....
7–24	100	170	9.0	5.93	10–24–32	192	203	7.7	3.33
8–3	110	186	8.9	5.63					

Antirachitic Effect of Sun-Cured Alfalfa Hay

Calf 216. This calf received the basal rachitic ration supplemented with sun-cured alfalfa. The results are given in Table 6. In view of the

TABLE 6

C–216. Effect of supplementing the rachitogenic ration with 2 pounds of sun-cured alfalfa hay

DATE	AGE	WT.	PLASMA		DATE	AGE	WT.	PLASMA	
			Ca	P				Ca	P
	days	*lbs.*	*mg. per 100 cc.*			*days*	*lbs.*	*mg. per 100 cc.*	
9–25–33	30	128	10.9	7.49	12–24–33	120	320	12.5	6.65
10–5	40	146	11.6	7.44	1–3–34	130	327	12.6	5.84
10–15	50	170	11.8	8.06	1–13	140	338	12.7	6.25
10–25	60	190	11.3	7.44	1–23	150	350	12.9	6.41
11–4	70	211	12.1	8.43	2–2	160	360	12.4	7.01
11–14	80	234	11.8	8.23	2–12	170	370	11.4	5.41
11–24	90	259	13.0	9.03	2–22	180	388	11.7	7.01
12–4	100	285	13.1	7.35	3–4	190	410	12.2	8.12
12–14	110	306	12.6	7.34	3–9[1]	195	421	12.5	7.27

[1] Slaughtered.

TABLE 7

Ash and mineral analyses of bones of calves fed the rachitogenic ration with various supplements

	8TH R. RIB				ORBITAL PLATE				DENTAL PAD			
Calf No.	*Ash* %	*Ca* %	*P* %	*Mg* %	*Ash* %	*Ca* %	*P* %	*Mg* %	*Ash* %	*Ca* %	*P* %	*Mg* %
					Timothy Ash							
C–144	41.46	16.58	7.23	0.53	43.25				46.69			
					Timothy—Sun Cured							
C–98	57.50	21.45	9.67	0.70								
C–98's	53.00	20.80	9.52	0.69								
C–100	60.65	23.05	10.30	0.38	59.25	23.00	10.88	0.88	54.10	20.76	10.18	0.84
C–109					49.70	18.98	8.87	0.81	47.42	18.49	8.53	0.51
C–148	42.89	17.65	7.69	0.57	36.81	14.58	6.62	0.68	58.44	18.14	7.78	0.89
					Timothy—Cured in Dark							
C–127	42.50	17.33	7.62	0.55	39.88				42.42			
C–133	47.68	18.98	8.06	0.51	46.07				48.74			
C–165	41.48	16.75	7.35	0.48	33.85	13.34	6.08	0.65	54.92	17.85	7.78	0.89
					Alfalfa—Sun Cured							
C–216	52.30	19.87	8.93	0.58	56.30	21.40	10.11	0.96				
C–252	55.10	21.35	10.28	1.01								

results secured from the feeding of timothy hay, the alfalfa was added at 8 days of age and limited to not more than 2 pounds per day in order to overcome anorexia. The plasma calcium and inorganic phosphorus values were normal throughout the duration of the experiment. No evidence of rickets was observed. The high calcium content of the alfalfa altered the

calcium to phosphorus ratio of the rachitogenic ration from 1.6:1 to 2:1 and 3:1. The high magnesium content of the alfalfa may also have aided in the prevention of rickets. The growth and condition of the calf at the time of slaughter was normal.

Calf C–252. The ration of this calf was supplemented with 1 pound of sun-cured alfalfa hay per day at 30 days of age. This amount of hay furnished enough of the antirachitic factor to maintain normal plasma calcium and inorganic phosphorus values up to 130 days of age. The skim milk was discontinued at 120 days of age which caused a temporary drop in the calcium and phosphorus intakes. The inorganic phosphorus dropped to 5.71 mg. per 100 cc. of plasma but as soon as the consumption of grain had increased sufficiently to meet the calcium and phosphorus requirements the plasma phosphorus quickly returned to normal. This indicates the importance of the level of calcium and phosphorus in the ration in studying the vitamin D requirements of young dairy animals.

DISCUSSION

Anorexia was one of the first symptoms observed in the calves used in this investigation to determine the antirachitic value of hay. Several calves lost their appetite for the basal rachitic grain mixture as well as for hay. Calf C–100 had consumed 15.4 pounds of sun-cured alfalfa hay from birth to 60 days of age and did not exhibit anorexia during her first year when 2 pounds of sun-cured timothy hay were the only source of the antirachitic factor. It is possible that anorexia was prevented in C–100 by feeding the sun-cured hay at an earlier age. On the other hand, C–98 had been raised to 90 days of age on a ration of sun-cured alfalfa and 10 cc. of cod liver oil per day in addition to the basal ration. This procedure did not prevent the occurrence of anorexia in this calf during the period of active rickets. C–148 was offered sun-cured timothy hay at 30 days of age but it refused to consume more than 1 pound per day which was ineffective in the prevention of anorexia. Most of the calves which were used in this investigation failed to consume the desired amount of hay.

Calf C–98 was cured of rickets with sun-cured timothy hay. The calf was limited to 3 pounds per day for some time but the marked improvement in the rachitic condition, as the result of the consumption of sun-cured hay, demonstrated the antirachitic potency of such a hay. Although this amount of sun-cured hay alleviated the rachitic symptoms in C–98 at 9 months of age, it failed to furnish enough vitamin D to prevent the reoccurrence of rickets at 15 months of age. After the second onset of rickets the hay was gradually increased to 6 pounds per day. This amount of hay furnished sufficient vitamin D to maintain the heifer, the development of the fetus and the average daily production of 27 pounds of milk. The results with calf C–100 indicate that 2 pounds of sun-cured timothy hay afforded ample protection against rickets up to about 1 year of age and that the gradual

increase in the hay consumption up to 6 pounds per day furnished sufficient vitamin D for maintenance and normal growth up to 2.5 years of age. Sun-cured alfalfa hay also had a marked antirachitic value as 2 pounds per day protected C–216 against rickets up to 195 days of age and 1 pound per day was effective in preventing rickets in C–252 up to 192 days of age. These results are in agreement with those reported by the Pennsylvania Station (21) where 2.5 pounds of sun-cured alfalfa protected calves from rickets while 1 pound prevented them from showing the clinical symptoms but failed to maintain normal blood inorganic phosphorus.

The calcium, phosphorus, magnesium and nitrogen balances in Table 3 demonstrate the antirachitic potency of sun-cured hay. Calf C–98 which received 3 pounds of hay per day as the only source of vitamin D had better utilization and storage of calcium and phosphorus than C–100 which received 2 pounds of hay per day.

After anorexia became pronounced the calves failed to make normal growth and in some cases growth ceased entirely or the animal lost weight. Some variations are shown, however, upon examination of the weights. The differences are probably due to individual variations in the ability of the calf to utilize the vitamin D supplied by the ration.

The results with the hay cured in the dark are of little value due to the manifestation of anorexia which prevented the consumption of sufficient hay to give it a fair trial.

Several types of rickets developed in the calves which were used in this investigation from the standpoint of blood analyses. The usual type was the low-plasma calcium, normal-inorganic phosphorus as indicated during the first attack of rickets of C–98. During the second attack, when sun-cured hay was fed, the condition was characterized by the normal calcium, low-inorganic phosphorus type. The latter type prevailed in the case of C–148, C–144 and C–100. The rachitic condition was characterized in C–133 and C–165 by the simultaneous drop in both plasma calcium and phosphorus. In cases where the first type of rickets prevailed, the inorganic phosphorus eventually became subnormal as the severity of the rickets increased.

The first alteration that appeared in the blood of the calves used in this investigation was a definite decrease in the concentration of calcium or inorganic phosphorus. When the Briggs modification of the Bell-Doisy technique is used, concentrations below 6.5 mg. per 100 cc. of plasma should be regarded as indicative of rickets, as the normal concentrations lie between 6.5 and 8.0 mg. for calves of the ages herein reported. It is quite probable that no rigid line can be drawn between the normal and pathological level of inorganic phosphorus because of individual variations and natural variations within the normal range. When the plasma calcium and inorganic phosphorus are determined at weekly intervals, the downward trend of either or both of these constituents preceded all other evidence.

The most significant features of the rachitic bones were their altered shapes, softer, less dense than normal, and their low ash and mineral content. The most outstanding observation, however, was the presence of pits in the articulating surfaces of the long bones of calves C–98 and C–100. A study of the pathology of rachitic calf bones will be reported in a subsequent paper.

SUMMARY AND CONCLUSIONS

1. Anorexia, which is usually associated with rickets in calves, was responsible for the lack of hay consumption under the conditions of this investigation.

2. It is difficult to use the curative method with calves when testing the antirachitic potency of roughages.

3. Two pounds of sun-cured timothy hay prevented rickets in calf C–100 up to 1 year of age and 3 pounds of sun-cured timothy cured rickets in C–98 at 9 months of age.

4. Two pounds of sun-cured alfalfa hay prevented rickets in calf C–216 up to 195 days of age and 1 pound of sun-cured hay protected C–252 up to 192 days of age.

5. The results indicate that the vitamin D requirement increases with age or size of the growing bovine and also that the requirement varies from individual to individual.

6. The calves used in this investigation showed three distinct conditions associated with rickets from the standpoint of blood analyses, (1) normal calcium, low phosphorus, (2) low calcium, normal phosphorus, and (3) low calcium, low phosphorus.

Addenda:

Since the acceptance of this paper for publishing G. C. Willis, L. S. Palmer and T. W. Gullickson reported that prairie hay may carry appreciable amounts of vitamin D.

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PERIOD OF LACTATION AND THE DIRECT TITRATABLE CHLORIDE VALUE OF MILK

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INTRODUCTION

The following types of natural milk may be high in chloride: (1) colostrum milk, (2) milk from diseased udders, and (3) milk from cows low in production, due to late lactation. In order to distinguish these three types from normal milk, a knowledge of the chloride content of the milk from healthy cows throughout the whole period of lactation is necessary.

The chloride test for detection of milk from diseased udders, particularly from cows having mastitis, has recently been used by Rosell (27), Hucker, Trudell and Jennings (9), Hayden (7), and others. Sharp and de Tomasi (30) have used the same test for a different purpose; namely, for the detection of milk from cows low in production, due to late lactation.

Eckles and Shaw (4) called attention to the fact that milk from cows late in lactation becomes bitter on standing. Palmer (16) (17) attributed this bitterness to the fatty acids liberated by the increased amount of lipase in the milk at the end of the lactation. Rogers (25), Rogers, Berg and Davis (26), Rice and Markley (21), Virtanen (37), Supplee (33), Nair (15), Dorner and Widmer (3), Sharp and de Tomasi (30), and others, have found that normal milk contains some lipase. Hammer and Bailey (6) and Koestler, Roadhouse and Lörtscher (12) called attention to the relation between bitter flavor, end of lactation, and milk high in chloride. The actual ratio of chloride to lactose was found to affect flavor by Gabathuler (5), Roadhouse and Koestler (24) and Roadhouse and Henderson (23).

In general, the more severely the milk secreting mechanism is injured by disease, or the longer a relatively small amount of milk is held in the udder, the more nearly the composition of the milk approaches that of the blood. This is evidenced by changes in the following milk constituents as shown by Storch (31) (32) Trunz (34), Eckles and Shaw (4), Koestler (10) (11) (13) and others: catalase, pH, titratable acidity, proteins, fat, lactose, pigments, sodium, potassium, calcium, phosphates and chloride: some exceptions occur. Colostrum milk has a relatively high titratable acidity, phosphate, and calcium content and a low pH. Both late lactation and mastitis milk show some of the same changes in composition and therefore respond alike to many tests for abnormality.

Many of the changes listed above have been suggested as a means of

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recognizing abnormal milk. Höyberg (8) found that the determination of the reaction (pH) of the milk colorimetrically with rosalic acid was a suitable method for recognizing the milk diseased udders. Baker and Van Slyke (2) studied the determination of the pH of abnormal milk colorimetrically and Baker and Breed (1) after comparing the use of rosalic acid, brom thymol blue, and brom cresol purple, recommended the latter. Brom thymol blue is now a more popular indicator, the use of which in detecting mastitis milk has been studied by Rosell (27), Udall and Johnson (35), Hucker, Trudell and Jennings (9), Plastridge and Anderson (18), and others. This test is often positive (greenish-blue) for late lactation as well as for mastitis milk, and as recorded by Plastridge and Anderson colostrum milk also changes this indicator to a greenish-blue color. The latter effect is due to an indicator error, since colostrum milk is notably low in pH. Gabathuler (5) suggested the use of the decrease in lactose, and Hammer and Bailey (6) the increase in chloride, determined by direct titration. Koestler (10) and his coworkers used what they called the chloride-lactose number which is 100 times the quotient obtained by dividing the amount of chloride by the amount of lactose

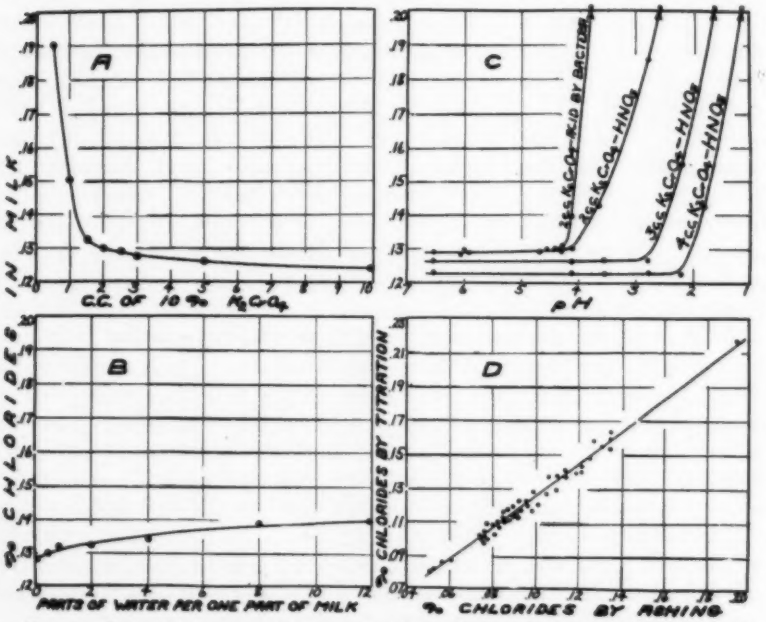

FIG. 1. Factors influencing the accuracy of the direct titration of milk for chloride. A, amount of indicator; B, dilution of the sample; C, acidity of the milk; D, amount of chloride as determined by ashing and by direct titration.

in the milk. The quotient changes proportionately more than either alone. Rosell (27) used changes in pH, chloride, catalase, and lactose to detect mastitis milk. Rullmann (28) and others found the leucocyte determination well adapted for the recognition of mastitis milk.

The direct titration of milk for chloride seems to be the simplest of the quantitative methods for the recognition of abnormal milk. Richmond (22) described the direct titration of milk with silver nitrate, using potassium chromate as an indicator, as a method for the detection of added salt. Hammer and Bailey (6) found that the values were uniformly about 0.026 per cent too high (Fig. 1, Part D), due to the effect of casein. Poetschke (19) (20) and Van der Burg and Koppenjan (36) criticized the direct titration method because of this error and the possible variation in it due to differences in protein. The data of Hammer and Bailey as plotted in Figure 1, Part D, show a definite linear relation, with only slight deviations, between the chloride determined by ashing and by direct titration of the milk. The variations due to the difference in protein would usually not be over $\pm$ 0.005 per cent of titratable chloride, except with colostrum milk. These considerations indicate that the direct titration of milk with silver nitrate would serve as a practical method for indicating any appreciable variation in chloride content. Rather than apply a correction factor, it is suggested that results obtained in this way be called *direct titratable chloride values*, bearing in mind that the true chloride content of the milk is less.

EXPERIMENTAL

Method of titrating

The procedure used in this paper was to transfer 18 grams of milk with a Babcock pipette to a 400 cc. beaker, add 2 cc. of a 10 per cent solution of potassium chromate, and titrate to a definite orange-red color with silver nitrate solution (8.624 grams per liter) of such a concentration that 1.00 cc. corresponded to 0.01 per cent of chloride in 18 grams of milk. Duplicate determinations usually fall within a range of $\pm$ 0.20 cc.

Some of the variations in titration procedure which influence the results were studied and are shown graphically in Figure 1. Part A shows that as the amount of indicator is increased the direct titratable chloride value rapidly decreases until about 1.5 cc. of potassium chromate has been added, and from then on an increase in indicator produces only a slight decrease in the value. Part B shows that the chloride value increases progressively with increasing dilutions of the milk. The effects of dilution and the amount of indicator show that liberties cannot be taken with the details of the titration if one expects to obtain comparable results.

It is well known that this method of titrating chloride does not work

if the solution is too acid. The pH limits below which the method ceases to be reliable were determined and are shown in Part C. The acidity in one case was produced by bacterial souring, in the others by the addition of nitric acid. Acidity causes no error so long as the milk has not curdled. If the milk has curdled two procedures are open, one the neutralization of the developed acidity and the other the addition of more potassium chromate indicator. If the latter procedure is followed 0.003 or 0.006 per cent should be added to correct for the effect of increasing the amount of indicator to 3.0 or 4.0 cc. It might have been better if 3.00 cc. of indicator had been selected for the standard procedure, but in order to make all of our data strictly comparable 2.00 cc. is still used.

The use of a yellow light lowers the end point somewhat, but does not appreciably sharpen it. Adsorption indicators were much less satisfactory than potassium chromate.

Morning and Evening Milking

Samples were taken from cows which were milked three times a day; at 5 a.m., 1 p.m., and 7 p.m. Only samples from the milkings at 5 a.m. and 7 p.m. were examined. On August 5, 1933, the milk from 29 cows was titrated; in 26 pairs the morning milk was the higher by the average amount of 0.007 per cent. On August 8, 1933, the milk from 40 cows was examined; in 39 pairs the morning milk was the higher; the average difference being 0.006 per cent. The cow which was the exception gave evening milk which was 0.001 per cent higher than the morning milk. This same cow was one of the three exceptions on August 5. The longer period between milkings in the case of the morning milk may account for the higher chloride value.

Sharp and de Tomasi (30) have presented a table showing the direct titratable chloride values of morning and evening milk obtained on successive days from a number of producers. The samples obtained from each producer were quite uniform unless the chloride value was high, in which case the value fluctuated greatly. This was probably due to cows which were milked once a day, some producers milking them in the morning, others in the evening. Variations in the severity of mastitis might also have played a part.

Udder Quarters and Fraction of Total Milking

Samples from the first, middle, and last portions of milk drawn from each of the four quarters of the udders of a number of cows were titrated. Data were obtained on three groups of cows; (1) normal in mid-lactation, (2) normal at the end of lactation and (3) cows in mid-lactation having mastitis. Results typical of the three types are presented in Table 1.

It will be noted that the chloride values of the milk from normal cows in mid-lactation are relatively low, and that the differences between the first, middle and last milk and between the different quarters of the udder are approximately within the experimental error of the titration method.

The uniformity between quarters and fractions of milk drawn tends to extend to the normal milk from cows late in lactation except the chloride values are higher. Some variation in the chloride content between the different quarters would be expected in late lactation since all quarters may not dry up at the same rate. The cows in late lactation were given a physical examination by a veterinarian to determine to what extent the high chloride values might be due to mastitis. Of special interest are the left

TABLE I

Examples of the direct titratable chloride values of the first (F), middle (M) and, last (L) portions of milk drawn from each quarter of the udders of normal, late lactation and mastitis cows

COW NUMBER	PORTION	LEFT		RIGHT	
		Front %	Rear %	Front %	Rear %
		Normal Milk			
1	F	0.099	0.101	0.099	0.101
	M	0.095	0.099	0.101	0.100
	L	0.102	0.106	0.099	0.106
2	F	0.089	0.094	0.090	0.090
	M	0.089	0.091	0.091	0.090
	L	0.091	0.092	0.093	0.090
3	F	0.099	0.098	0.099	0.101
	M	0.101	0.098	0.098	0.101
	L	0.101	0.099	0.098	0.101
4	F	0.103	0.104	0.106	0.105
	M	0.103	0.104	0.105	0.105
	L	0.103	0.105	0.105	0.104
		Late Lactation Milk			
5	F.	0.126	0.128	0.128	0.125
	M	0.123	0.129	0.127	0.125
	L	0.125	0.126	0.128	0.127
6	F	0.172	0.139	0.162	0.174
	M	0.128	0.133	0.134	0.159
	L	0.130	0.131	0.138	0.160
7	F	0.143	0.212S	0.131	0.138
	M	0.148	0.148	0.135	0.137
	L	0.153	0.153	0.150	0.141
8	F	0.223	0.237D	0.212	0.153D
	M	0.194	0.166	0.222	0.166
	L	0.211	0.177	0.230	0.178
9	F	0.186S	0.165S	0.174S	0.194S
	M	0.157	0.157	0.143	0.148
	L	0.139	0.134	0.144	0.148
10	F	0.192	0.187	0.217	0.185
	M	0.192	0.187	0.200	0.187
	L	0.199	0.189	.....	0.188

TABLE I—(*Continued*)

COW NUMBER	PORTION	LEFT		RIGHT	
		Front %	Rear %	Front %	Rear %
		Mastitis Milk			
11	F	0.166	0.183	0.235	0.181
	M	0.129	0.166	0.177	0.141
	L	0.142	0.197	0.257	0.161
12	F	0.136	0.182	0.139	0.114
	M	0.115	0.141	0.132	0.115
	L	0.126	0.153	0.142	0.125
13	F	0.275	0.256	0.187	0.295
	M	0.121	0.118	0.116	0.120
	L	0.132	0.119	0.119	0.122
14a	F	0.143	0.126	0.101	0.100
	M	0.105	0.104	0.098	0.098
	L	0.126	0.122	0.100	0.100
14b	F	0.170	0.127	0.098	0.101
	M	0.104	0.102	0.095	0.095
	L	0.115	0.112	0.094	0.096
14c	F	0.132	0.110	0.107	0.109
	M	0.107	0.108	0.100	0.102
	L	0.130	0.121	0.101	0.103

S = slight mastitis. D = distinct mastitis.

front and right front quarters of cow number 8. The chloride was higher in these two quarters than in the other two, yet these quarters did not show evidence of mastitis, while the other two showed distinct evidence of it. The chloride content, even though high, was fairly uniform in the three parts of the milk drawn from these two non-mastitis quarters.

The cows in the mastitis group were in the middle of lactation. The milk from cow 14 was titrated on three successive days, as indicated by a, b, and c. Right front and rear quarters were free from mastitis, the other two showed evidence of it. The chloride values in the two mastitis quarters fluctuated considerably from day to day and in the different portions of the milk.

Ordinarily the first and last milk drawn from quarters infected with mastitis is high in chloride and pH, and low in titratable acidity, while the middle milk is often normal in chloride, pH and titratable acidity. A few exceptions were found when production was very low and when the milk was stringy. This lack of uniformity usually permits one to distinguish cows giving milk high in chloride due to mastitis from those giving high chloride due to late lactation. The fraction of the milk drawn is important in testing for mastitis. In several of the mastitis group of cows, if the first milk drawn were tested, very severe mastitis would be indicated, while if the middle milk alone were examined the report might be that the chloride, pH, and titratable acidity tests for mastitis were negative. The first streams should be selected in testing for mastitis.

Stage of Lactation

During the years 1932 and 1933 the milk from a number of healthy cows was titrated monthly for chloride. In the group were included 13 Jerseys, 8 Guernseys, 15 Holsteins, 4 Brown Swiss, 5 Ayrshire and 4 Shorthorns. In all 326 samples were examined. The cows were examined regularly by veterinarians and all samples from cows showing active mastitis were eliminated. Furthermore if the chloride values began to increase unexpectedly, and later active mastitis was recognized, the data from such cows were excluded. This observation in regard to the detection of mastitis agrees with that of Rosell (27) who found that the early stages can be detected best by chemical and less readily by bacteriological means. In Figure 2 the titratable chloride values are plotted against the fraction of the total lactation period.

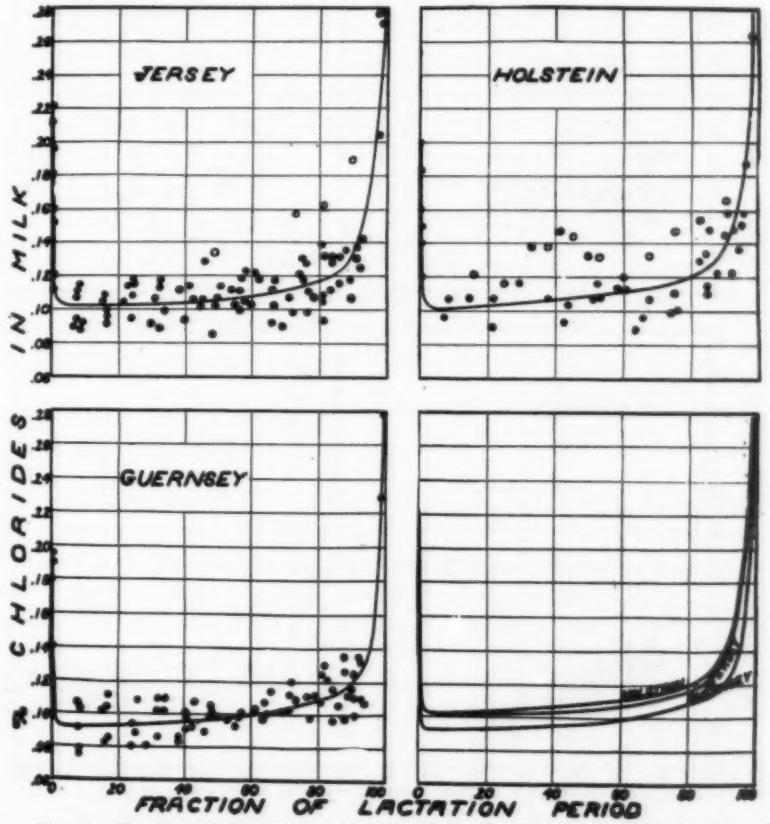

FIG. 2. Fraction of the lactation period and the direct titratable chloride value of the milk of different breeds.

Colostrum milk gave quite high chloride values, but the value fell rapidly so that at the end of one to two weeks a minimum was reached. The chloride increased very slowly during the next 60 per cent of the lactation period and from there on more rapidly until a very abrupt rise occurred after 90 per cent of the period of lactation had been completed. The data indicated by circles were obtained from one or two cows. These cows were repeatedly pronounced free from mastitis. They were outstandingly hard milkers.

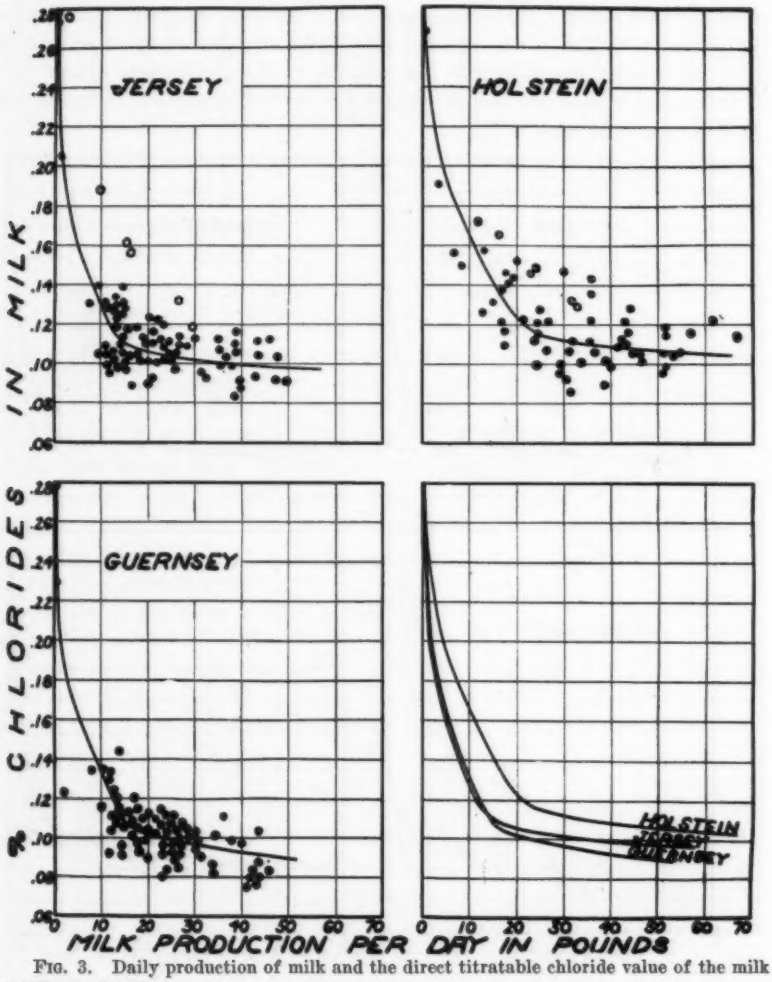

FIG. 3. Daily production of milk and the direct titratable chloride value of the milk of different breeds.

Production

In Figure 3, the direct chloride titration values are plotted against the average daily production. The results show a striking relation between the two factors. The chloride values begin to rise rapidly after the production falls below about 15 pounds for the Jerseys and Guernseys, and below about 25 pounds for the Holsteins. This relationship is of service as indicating whether high chloride values are due to late lactation or to a diseased condition in the udder. The importance of the relation to production was shown by a Holstein cow which still gave about 25 pounds of milk after being milked continuously for about five years. The chloride value was 0.13 per cent.

Breed Difference

The data indicate quite clearly that Holstein milk tends to be higher in chloride than does Guernsey or Jersey milk. This is probably a true breed difference and is not due to a systematic error, since the known errors point in the reverse direction. The interfering proteins are higher in the Guernsey and Jersey milk. The cows had all been scored for severity of mastitis according to a system devised by Udall and Johnson (35) on a basis of 1 to 4, the score 1 indicating little or no evidence of mastitis and 4 indicating the active condition. According to this classification the average scores were as follows: Guernsey 1.96, Jersey 1.93 and Holstein 1.73. Sharp (29) reported a correlation of -0.603 ± 0.013 between titratable acidity and titratable chloride content of over 1000 samples of market milk. This correlation is in line with the breed difference, but was undoubtedly influenced by the presence of milk from cows late in lactation and having mastitis.

Effect of Gestation

The effect of gestation on the chloride value is shown in Figure 4. The chloride is plotted against the months of gestation in Part D, and the expected increase with gestation is shown. The same data are plotted against months of lactation and give a curve which is more irregular because of the variation in the lactation periods. The curves in Part B indicate that although the chloride content increases as the lactation period advances, the progress of gestation tends to negate, to some extent, the increase in titratable chloride. This effect is also indicated in Parts A and C. At the same level of production the chlorides are lower the more advanced the period of gestation. This result suggests the possibility that the chloride content of the blood of the cow might decrease as the gestation advances. Such a decrease in chlorides in the blood would be in line with the increase in blood volume as found by Miller (14). Other explanations such as a change in the secreting mechanism in the udder are possible.

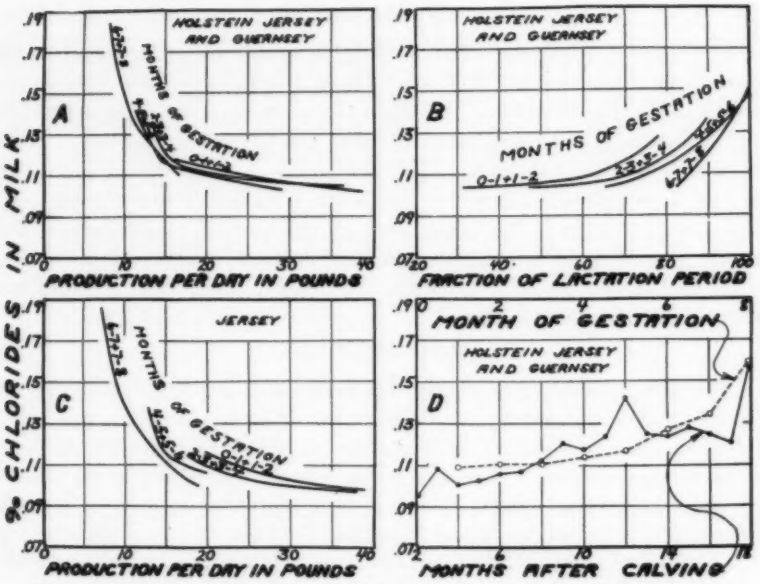

FIG. 4. Period of gestation and the direct titratable chloride value of the milk.

SUMMARY AND CONCLUSIONS

The following statements refer to the chloride value of milk as determined by the direct titration of the milk with silver nitrate, using potassium chromate as an indicator.

1. The direct titratable chloride value of milk was found to increase with the dilution of the milk and with decreased amounts of indicator. As the acidity of the milk increased, no influence upon the end point was found until after the casein had coagulated.

2. Differences between normal morning and evening milk were slight.

3. The fraction of the total milking of the quarter of the udder and the different quarters themselves had little effect on the chloride values of the normal milk from healthy cows.

4. The first and last milk drawn from quarters infected with mastitis was high in chloride and pH and low in titratable acidity, the middle portion often approached or fell within the range of normal mastitis-free milk. Wide variations were found between the different quarters in the case of cows infected with mastitis.

5. The chloride in milk from healthy cows fell very rapidly during the first few days of lactation, reached a minimum, increased slightly during the first 60 per cent of the lactation period, and then increased more rapidly, particularly during the last 10 per cent of the period.

6. A definite relation was found between the chloride value and the daily production of milk from healthy cows. A marked increase in chloride began when the production of Jersey and Guernsey cows fell below 15 pounds, and when the production of Holstein cows fell below 25 pounds of milk a day.

7. The milk from healthy Holstein cows tends to be higher in chloride than does the milk from healthy Jersey and Guernsey cows.

8. Gestation exerts a negating tendency on the increase in chlorides due to progressive lactation.

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THE ADVANTAGES OF SKIM-MILK AGAR FOR THE DETERMINATION OF THE SANITARY QUALITY OF MARKET MILK

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Attention was called by Sherman (1) to the advantages in using an agar containing a fermentable carbohydrate in the bacteriological control of market milk. He presented data which showed that the colonies of bacteria normally present in milk were two to ten times larger on lactose agar than on plain agar. He made comparative bacterial counts on plain and lactose agar which showed an average increase, in favor of lactose agar, of 43 per cent on raw milk and 378 per cent on pasteurized milk. Sherman mentioned that "glucose is apparently just as efficient as is lactose, both as to number and size of colonies," and that "since glucose is cheaper it should perhaps be recommended as the standard for routine work." He also suggested that the amount of sugar could be reduced to 0.1 per cent "without impairing the value of the medium." Simmons (2), and others, have confirmed Sherman's findings.

Ayers and Mudge (3) suggested a milk powder agar for use in determining the numbers and kinds of bacteria in market milk and other dairy products. They found that milk powder agar gave from 3 to 75,000 per cent higher counts than those obtained on plain agar. The colonies were larger and consequently could be counted with greater ease and accuracy. The casein agar reported by Ayres (4) was found to be unsatisfactory unless incubation periods longer than two days were employed.

The modification of milk powder agar suggested by Zoller (5) was found by Norton and Seymour (6) to be a definite improvement. This modified milk powder agar gave higher counts than any other medium tested by these workers, but it was criticized because of the complicated procedure involved in its preparation.

EXPERIMENTAL

The results here reported include data obtained on approximately 760 samples of milk taken from the milk supply of 250 dealers located in various parts of the State of New York. These milk samples were taken three times during the year at periods representing winter, spring, and summer conditions. The samples of milk were plated in duplicate, in dilutions of

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1–100 and 1–1,000, on standard nutrient agar and on a milk agar having the following composition:

Fresh raw skim milk	2.0 per cent
Bacto peptone	0.5 per cent
Bacto beef extract	0.1 per cent
Glucose	0.1 per cent
Agar	1.5 per cent

This medium is a modification of one employed with good results by J. M. Sherman (unpublished data) almost twenty years ago, in which he used 0.2 per cent skim milk powder, 1 per cent peptone, and 1.5 per cent agar. In the medium here reported, skim milk was used instead of milk powder with the belief that it would be more uniform in consistency, freer from sediment, and would give uniformly high bacterial counts. Small amounts of beef extract and glucose were added on the assumption that they might improve the nutritive value of the medium, and support the luxuriant growth of certain types of bacteria which, in the absence of these constituents, might grow very poorly or not at all. A medium of the above composition is simple, easily made, and should cost as little or probably less than the present plain agar suggested by Standard Methods.

Ayers and Mudge (3) say "attempts to make a medium by using skimmed milk, with its casein dissolved, and adding peptone and beef extract were not successful." If much larger amounts of skim milk, or phosphate buffers, are added to the medium, precipitation will occur. The required amounts of glucose and skim milk may be added to the adjusted, autoclaved, and filtered nutrient medium just previous to its final sterilization. If the medium previous to the addition of milk is adjusted to pH 7.0, the reaction of the completed medium will be approximately pH 6.5. If thought advisable, the pH could be raised by the addition of sodium hydroxide.

Table 1 shows the general trend of the results obtained from the comparative counts made on the three sets of samples. These data are reported

TABLE 1

Average percentage increase in favor of skim-milk agar between counts made on standard agar and on skim-milk agar

KIND OF MILK	WINTER SAMPLES FEB 6–7		SPRING SAMPLES MARCH 28–29		SUMMER SAMPLES JUNE 6–7	
	No. tested	Per cent increase	No. tested	Per cent increase	No. tested	Per cent increase
Pasteurized	221	127	199	351	198	196
Raw	25	5	56	16	56	18

separately because of the extreme differences in temperature when the tests were made. Many extremely high counts were observed during the warmer

weather. Frequently neither set of plates could be accurately counted on the highest dilution used, which probably tended to obscure many of the existing differences in counts. On the average, the skim-milk agar count on pasteurized milk was two to four times as large as the corresponding count on standard agar. A slight increase in favor of skim-milk agar was also observed on the smaller number of samples of raw milk tested.

The presence in pasteurized milk of certain bacteria which are unable to grow on standard agar is the obvious reason for higher counts on skim-milk agar. That varying numbers of such bacteria are often found in pasteurized milk is well known. Probably the presence of these types of bacteria explains the results reported in Table 2.

TABLE 2

Some striking differences in counts from pasteurized milk on standard agar and on skim-milk agar

NUTRIENT AGAR COUNT	SKIM-MILK AGAR COUNT	DIFFERENCE IN FAVOR OF SKIM-MILK AGAR
		Per cent
7,900	1,230,000	15,470
2,400	73,500	2,962
29,900	815,000	2,626
2,100	57,300	2,152
5,600	46,500	730
14,900	117,000	685
19,500	119,000	510
28,500	123,000	332
2,400	9,300	287
3,500	10,300	190

It will be observed from the distribution of percentage differences reported in Table 3 that 60 per cent of the 608 pasteurized milk samples showed a definitely higher count on the skim-milk agar; about 35 per cent

TABLE 3

Relative distribution of percentage differences in counts from pasteurized milk on plain agar and skim-milk agar, using plain agar as standard

RANGE IN PERCENTAGE	NUMBER OF SAMPLES
−26 per cent to −60 per cent	28
−25 per cent to − 1 per cent	70
0 per cent to 25 per cent	145
26 per cent to 200 per cent	274
201 per cent to 500 per cent	50
501 per cent to 2000 per cent	33
2001 per cent to 5000 per cent	5
Over 5000 per cent	3
Total	608

gave approximately the same count (−25 per cent to +25 per cent); and less than 5 per cent of the samples tested gave a lower count on the skim-milk agar. It is obvious that the difference between the counts on different samples is far from consistent. This very inconsistency brings out most clearly the advantages of the skim-milk medium.

Since logarithms of bacterial counts tend to minimize small differences which might be considered significant and also offer an opportunity to present large groups of data in a small space, the logarithms of the bacterial counts for each period have been plotted in Figures I, II, III, and IV.

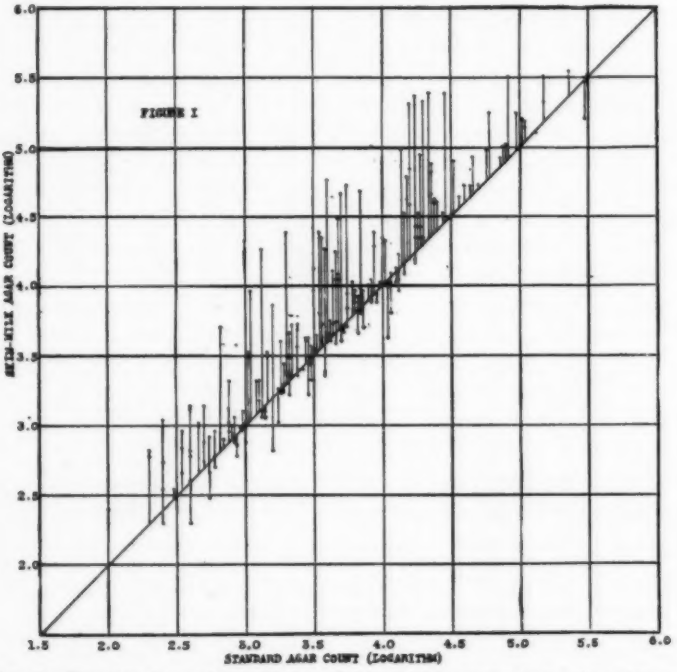

FIG. I. The logarithm of the number of bacteria *in pasteurized milk* which grew on standard agar and on skim-milk agar.

The standard agar counts are plotted on the diagonal line.

Samples taken February 6 and 7.

If the skim-milk agar count exceeds the plain agar count, its logarithm appears above the diagonal line; if it is less, it appears below this line. The distance vertically from each dot to the diagonal line represents a single logarithmic difference. In certain cases, several of these differences are plotted on the same vertical line. This means that the standard agar counts

on several samples were the same, whereas the skim-milk agar counts varied. Each dot represents one skim-milk agar count. The logarithms of these comparative counts on pasteurized milk appear in Figures I, II, and III. A glance at these graphs shows that the bulk of the skim-milk agar counts are higher than the standard agar counts. The logarithmic differences on certain samples are conspicuous. The general trend of the three charts is similar, although the counts on both media tend to be higher as warmer weather approaches.

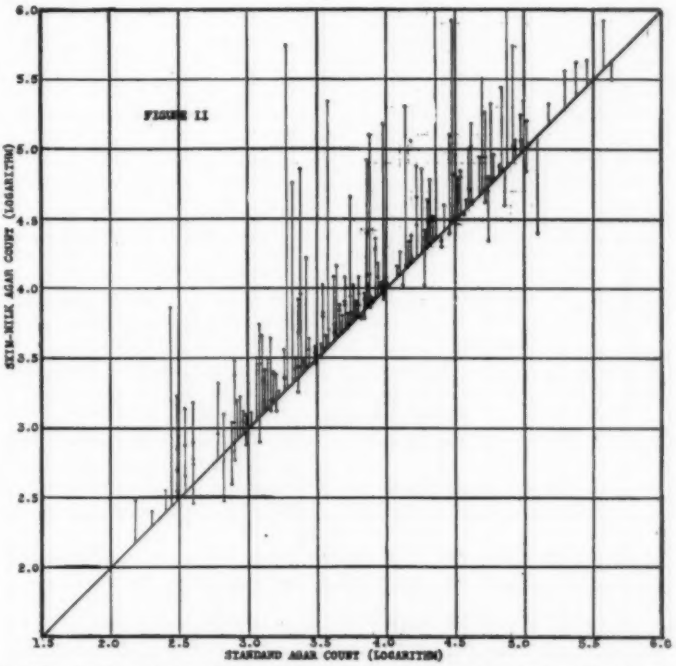

FIG. II. The logarithm of the number of bacteria *in pasteurized milk* which grew on standard agar and on skim-milk agar.

The standard agar counts are plotted on the diagonal line.

Samples taken March 28 and 29.

Because of the small numbers of raw milk samples tested, the results for the three periods are presented together in Figure IV. The logarithmic differences in these counts on raw milk are believed to be of but little significance.

These data have been analyzed according to the percentage difference in counts, based on logarithmic averages as employed by Robertson and Frayer (7). The logarithmic averages of the standard and skim-milk agar

counts on pasteurized milk and the percentage differences calculated from these averages are presented in Table 4. Similar calculations were made

TABLE 4

Theoretical percentage differences in counts from pasteurized milk based on logarithmic averages

	LOGARITHMIC AVERAGES		DIFFERENCE IN COUNTS: PERCENTAGE
	SKIM-MILK AGAR COUNTS	STANDARD AGAR COUNTS	
			Per cent
Winter samples			
Feb. 6–7	3.787	3.585	59
Spring samples			
Mar. 28–29	4.024	3.804	66
Summer samples			
June 6–7	4.616	4.366	78

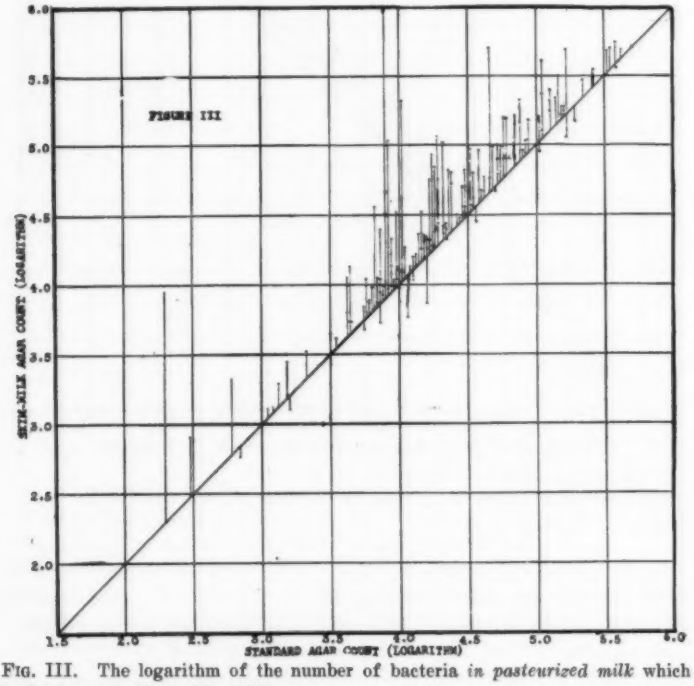

FIG. III. The logarithm of the number of bacteria *in pasteurized milk* which grew on standard agar and on skim-milk agar.

The standard agar counts are plotted on the diagonal line.

Samples taken June 6 and 7.

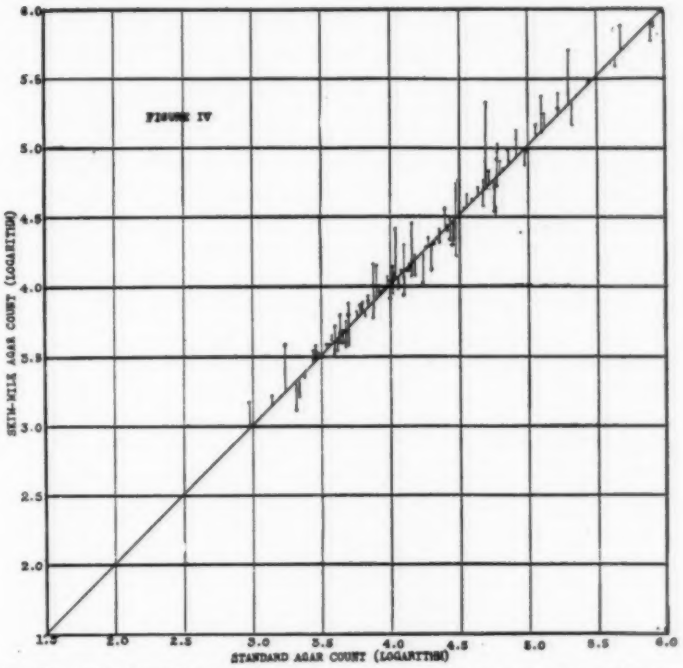

FIG. IV. The logarithm of the number of bacteria *in raw milk* which grew on standard agar and on skim-milk agar.

The standard agar counts are plotted on the diagonal line.

Samples taken February 6 and 7; March 28 and 29; and June 6 and 7.

on the raw milk data, but were found to have but little significance. For each set of logarithmic averages the percentage difference in count was calculated from the theoretical plate counts corresponding to these averages. This is illustrated for the spring samples of pasteurized milk:

Logarithmic average of skim-milk agar count is 4.024, which is equivalent to 10,570 bacteria per cc.

Logarithmic average of standard agar count is 3.804, which is equivalent to 6,370 bacteria per cc.

$$\frac{10{,}570 - 6{,}370}{6{,}370} \times 100 = 66$$ per cent theoretical percentage difference in counts.

So great a percentage difference in counts, when based on logarithmic averages, is believed to be truly significant.

SUMMARY

The fermentable carbohydrates and other milk constituents in skim-milk agar make it a desirable medium to use in the routine control of market milk and other dairy products.

The skim-milk agar counts on 618 samples of pasteurized milk were, on the average, two to four times as large as the corresponding counts on standard agar. The counts on 137 samples of raw milk were only slightly higher.

The colonies were much larger and consequently could be counted with greater ease and rapidity.

The slight opacity of the medium prevents the glare often experienced when artificial lighting devices are used. Acid-producing and protein-digesting types of bacteria can be differentiated on this medium.

It supports the growth of bacteria responsible for mastitis in cows.

It is simple and easy to make and no more expensive than the present standard agar.

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VITAMIN A CONTENT OF PASTURE PLANTS

11. TIMOTHY (*PHLEUM PRATENSE* L.) AND RED TOP (*AGROSTIS ALBA* L.) UNDER PASTURE CONDITIONS AND FED GREEN*

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The public constantly demands higher quality in dairy products, and progressive dairymen attempt to meet such demand. In recent years, because of the consumer's interest in the vitamin content of milk, investigators, and even some commercial milk producers, have tried by various methods to fortify the vitamin content of milk. After studying the problem, Krauss (12) summarized the situation as follows: "Vitaminization and mineralization of foods probably can not be justified except where natural foods fail to furnish these vital factors. This is especially true of milk."

Although there is considerable sentiment against making milk a "drug store product" by adding material to it, nevertheless, public demand will insure continued interest in milk of high vitamin content whether produced from natural feeds or treated in some manner.

Milk, butter, and cheese are important sources of vitamin A in the human diet. Many investigators have suggested that the vitamin A content of milk might be greatly influenced by the vitamin A content of the feeds consumed by the cow. In a previous paper from this station (23) some of the earlier results published on this subject were reviewed. Later investigations (1, 3, 4, 6, 13, 14) have confirmed this conclusion. It has been shown by Moore (19) that the cow is capable of converting the carotene in her ration into vitamin A, but the daily output of carotene and vitamin A in the milk fat is very small compared with the carotene intake, never exceeding that of normal summer butter regardless of the amount in the feeds consumed. Baumann, Steenbock, Beeson, and Rupel (2) report: "When the carotene intake of the cow was increased, there was an increase in both the carotene and vitamin A content of the butterfat. Calculations indicated that 3.3 per cent of the vitamin A ingested on a low carotene ration was secreted into the milk; on a high carotene ration only 1.3 per cent was secreted."

In the past, vitamin A has not been considered a limiting factor in the well-being of cattle, but more recent results indicate that under some conditions it may be. Hart and Guilbert (9) report vitamin A deficiency in range cattle under natural conditions. Deficiency developed when the dry

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feed season was unusually prolonged. Manifestation was more severe when the diet was otherwise complete and supplied in amounts above maintenance. Reproductive failure and dead, weak, or sick calves were the most common results.

Meigs and Converse (15) conclude from their experiments that "In the case of liberally milking cows fed on grain and hay or on grain, hay, and silage, without pasture, the ration is likely to be deficient in vitamin A unless it contains a large proportion of legume hay of good quality. Young calves from birth to the age of six months are highly susceptible to A deficiencies." The same authors (14) report that when timothy hay of mediocre or low quality was fed to dairy cows reproduction was reduced, general health of the cows lowered, milk production decreased, and pregnancy was produced with more difficulty.

Woodward and Nystrom (24) in their bulletin on feeding dairy cows, point out that vitamin A is the vitamin most likely to be deficient in the ration of the dairy cow, and that as a result of this deficiency cows may give birth to weak, dead, or premature calves. They believe the quantity of milk will not be reduced so much as the vitamin A value. Because of the reserve supply in the liver, the cows themselves may go for months without showing the bad effects of the deficient ration, but calves fed the milk from such cows will cease to grow and soon will die if they are not given supplementary feeds rich in vitamin A or carotene.

Storage of vitamin A in the body of the cow may be sufficient to take care of other body functions for several months, but Hilton, Hauge, and Wilbur (11) found that as far as milk production is concerned the vitamin A value of butter responds very rapidly to changes in the vitamin A value of the rations fed to the cows and that it is possible to maintain a high vitamin A value in butter by practical feeding methods. Fraps, Copeland, and Treichler (8), however, from their study of the vitamin A requirements of dairy cows conclude that : "silage and ordinary hays and fodders apparently will not supply enough vitamin A potency to maintain a high content of the butterfat. Green growing pasture grasses appear to be needed to maintain the production of butterfat high in vitamin A."

Dutcher (5) calls attention to the fact that the vitamin content of leafy plants and vegetables may be correlated with greenness, metabolic activity, and maturity. In discussing the growth response from vitamin A and its precursor, carotene, he sums up the problem as far as animal feeding is concerned as follows: "It is probable that we shall find that some of our so-called 'vitamin A-rich' foods are really quite deficient in this vitamin, *per se*, but that these foods are really rich in the parent substance, carotene. From the nutritional and clinical standpoints this fact need not cause concern so long as the fat-soluble factor is rendered available for the animal organism."

Investigation of the vitamin A activity of the most common pasture plants seems justified because, first, the vitamin A activity of the feed consumed by the cow affects the vitamin A activity of the milk and butter, which is important in human nutrition; second, pasture represents the major portion of the cow's feed during nearly half the year; and third, significant differences in the vitamin A activity of different plants may indicate consideration of this factor along with others in formulating pasture mixtures for dairy cows.

Very little has been reported on the vitamin A activity of forages. In a previous paper from this Station (23), the vitamin A activity of white clover (200 rat units per gram) was reported to be about twice that of Kentucky bluegrass.

EXPERIMENTAL PROCEDURE

The method used for determining the vitamin A content of these plants was based upon the technique of Sherman and Munsell (20). Young albino rats, 21 to 28 days old and weighing from 32 to 51 grams, were taken from mothers who had received a ration consisting of 62 per cent ground whole wheat, 30 per cent whole milk powder, 4 per cent wheat germ, 3 per cent powdered egg yolk, and 1 per cent iodized salt. These rats were placed on a basal vitamin A-free diet which consisted of 67 per cent cornstarch, 18 per cent air-heated casein, 10 per cent dried yeast powder, 1 per cent sodium chloride, and 4 per cent salt mixture (Osborne and Mendel (17)).

To free the ground commercial casein of vitamin A, it was treated by the heat method suggested by Potter (18). Three hundred gram quantities were spread on shallow trays to a depth of $\frac{3}{4}$ inch and heated in a Freas oven at 110° C. for seven days. The casein was stirred twice daily to secure better exposure. After heating it was washed twice with distilled water and allowed to dry. Vitamin D was furnished by feeding one drop of Mead's Viosterol three times a week.

At the end of three weeks of the depletion period the rats were put in individual cages made of $\frac{1}{2}$ inch wire screen. From the time the rats were put in individual cages to the end of the depletion period they were weighed every other day.

As soon as there was a cessation of growth or the first sign of ophthalmia (slight swelling of the eyelids and an accumulation of exudate on the cornea), usually accompanied by a barely perceptible tendency to flabby musculature and slightly unkempt fur, the depletion period was ended and the test period begun.

Fresh green samples of timothy and red top under pasture conditions were received twice a week from the Caldwell Substation. To prevent moisture losses in shipping, the grass was wrapped in cellophane or paper sacks and placed in paper cartons. Upon arrival, each sample was placed

in a half-pint Economy fruit jar and kept in the refrigerator. Practically all the samples consisted of basal foliage and contained very few stems. Timothy had an average moisture content of 68 per cent for all feedings, while red top contained 61 per cent moisture.

The grasses were fed three times a week as supplements to the vitamin A-free diet. Timothy was fed at the rate of 10 and 12½ milligrams per day, while red top was fed at the rate of 10 and 7½ milligrams. Litter mates were used for comparisons between grasses, and a negative control from each litter was continued on the basal diet until death.

Weekly records of weights and food consumption, and careful notes on the condition of the animals were kept for eight weeks. The method of eye scoring was that used by Steenbock and Wirick (22). All animals were autopsied at death or at the end of the experimental period.

RESULTS

At the beginning of the depletion period, the average weight of the rats in all groups was 42 grams, and at the end of the depletion period 98 grams. The average length of the depletion period was 26 days.

Average growth response of the rats being fed each of the grasses is shown in Table 1 and Figure 1. The experiments here reported were planned and carried out on the basis of the eight-week test period used by Sherman and Munsell (20), but results are presented for periods of four weeks (28 days). A 1934 revision of the "Text and Assays for Cod Liver Oil" of the Pharmacopoeia of the United States prescribes an official method of assay for vitamin A (16). Specifications for the depletion period, assembling of rats in groups, and recording of data are given. Concerning the validity of data the method reads as follows: . . . "The data from an assay group shall be considered valid for establishing that an assay oil conforms with the U. S. Pharmacopoeia standard for Vitamin A in cod liver oil only when two-thirds or more but not less than six rats shall have made individually between the beginning day of the assay period and the twenty-eighth day thereafter an increase in body weight which shall equal or exceed 12 grams." This rule has been used to establish the validity of data from an assay group for vitamin A potency of pasture plants. No rat which has not made a gain of 12 grams or more in four weeks has been used to establish average performance of an assay group. The number of rats in each group and the number used in the average are indicated in the table. The results can not be expressed in International Units of Vitamin A, however, because there are no data from a group fed a "Reference Cod Liver Oil." The value is calculated, therefore, in rat units per gram from the daily dose in milligrams which would give an average gain of 12 grams in 28 days.

Animals (10 rats) receiving 60 milligrams of timothy per week made

TABLE 2

Average growth response of rats when fed fresh timothy and red top as vitamin A supplements

PASTURE PLANT	SOURCE	DATE OF TEST PERIOD	AMT. OF PLANT FED WEEKLY	RATS FED	RATS USED		AVE. WT. AT BEGINNING OF DEPLETION PERIOD	AVE. WT. AT BEGINNING OF TEST PERIOD	AVE. WT. AT END OF 4 WEEKS TEST PERIOD	AVE. GAIN IN WT. AT 4 WEEKS	COEFFICIENT OF VARIATION	RAT UNITS OF VITAMIN A
			gms.			%		*gms.*	*gms.*	*gms.*		
Timothy	Caldwell	5–16 to 7–11	0.060	12	10	83	43	100	126	26 ± 1.5	26	220 ± 13
"	"		0.075	4	4	100	39	101	124	23	*	*
Red Top	"	"	0.060	12	10	83	42	98	135	37 ± 1.1	15	308 ± 10
" "	"	"	0.045	4	4	100	39	99	124	25	*	*
Negative Controls		"	0.000	8			43	91	average survival—15 days average loss in weight—15 grams			

* Insufficient data.

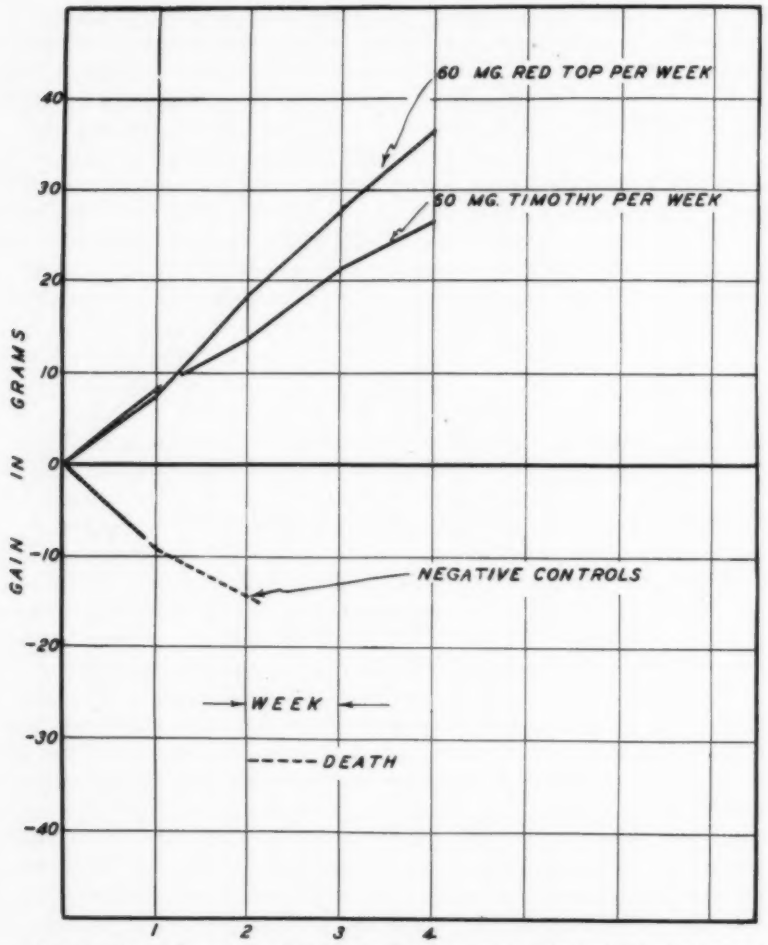

FIG. 1. Average weekly weight increases of rats, previously depleted of vitamin A, when fed either timothy or red top as a supplement to a vitamin A free diet.

Average weekly losses in weight of negative control rats were computed each week by averaging the weight of all living rats as long as 50 per cent of the original group survived. The line was terminated by extending it directly from the last week showing 50 per cent survival to a point indicating the average survival days and average weight at death for the entire group. The broken line indicates that one or more deaths occurred during the week.

an average total gain in body weight of 26 ± 2 grams in four weeks. This would indicate that this grass contained 220 ± 13 rat units of vitamin A activity. The average total gain of the 10 rats fed 60 milligrams of red top per week was 37 ± 1 gram. This would indicate 308 ± 10 rat units of vitamin A activity per gram in red top.

The coefficient of variation in the group fed timothy, 60 milligrams per week, was 25 per cent, while in the group fed red top, at the same level, was 16. The variability of these results compare favorably with the findings of Sherman and Burtis (21), who report that when the animals were classified by sexes the coefficient of variation was 32 per cent at the end of the fifth week at a vitamin intake level twice that needed for a growth response of 3 grams per week and 45 per cent at a level inducing 3 grams gain per week.

At the other level on each grass, the great variation found within the group, together with the limited number of rats (less than six), would preclude conclusions from these groups other than to substantiate the dependability of the levels used in interpreting results.

The difference between the rat units of vitamin A activity in red top, 308 units, and timothy, 220, was 88 units. The probable error of the difference was 11 units, or the reliability of the difference may be expressed as 88 ± 11. Since the difference between the rat units in the two pasture plants (88) was eight times the probable error of the difference, the difference is a true one.

In order that the results obtained may be compared with data reported on the basis of 24 grams gain in eight weeks, the average gain made by each group in eight weeks is given. When the average gain for the longer period is the basis used for calculating the rat units per gram, all animals in a group are usually included whether or not they are gaining at the end of the test. All animals, therefore, are used in summarizing the eight-week averages. On the 60-milligram per week level the 12 animals fed timothy gained 39 ± 1.2 grams in eight weeks, indicating a value of 163 ± 5 rat units per gram; while the 12 rats on red top gained 48 ± 3.4 grams, or 200 ± 15 rat units per gram. All the animals receiving 60 milligrams of red top per week were free of xerophthalmia at the end of four weeks and also at end of eight weeks. Only one of the animals of the group used with the timothy showed eye infection at four weeks, and this condition failed to clear by the end of eight weeks. One of the other rats included in the longer test group on timothy also failed to develop normal eyes. Post-mortem examinations of the animals at the end of eight weeks revealed some signs of infection, especially in the ear and nasal sinuses. The eight animals used as negative controls survived an average of 15 days beyond the end of the depletion period and lost an average of 15 grams in body weight. Post-mortem examination of these animals showed that 3 had pus in the

nasal sinuses, all had pus in the inner ear, 2 had pus at the base of the tongue, 2 had congestion in the intestinal tract, and 1 had pus in the lymph glands.

DISCUSSION

White clover and Kentucky bluegrass previously have been reported to contain 200 and 100 rat units, respectively, of vitamin A activity when computed from data covering a feeding period of eight weeks (23). When computed from gains made in the first four weeks, as used in this paper, the white clover contained 242 ± 19 rat units and the Kentucky bluegrass 175 ± 11. In comparable units, timothy with 220 ± 13 would rank between bluegrass and white clover. Red top with 308 ± 10 units contained about one-third more than even white clover. These results indicate that different pasture plants may vary considerably in their content of vitamin A activity. Although the 4 weeks test period seems to have some advantages over the 8 weeks period, it appears that higher vitamin A rating may be expected from the same feed level.

The fact that red top contained about 50 per cent more rat units of vitamin A activity than timothy may be attributed to the possibility of a higher carotene content. Although no tests for carotene were conducted, and each grass was green when fed, some justification for this assumption is indicated by the fact that red top takes on a reddish cast when mature while timothy does not.

Although many writers have suggested that fresh green grass is probably a potent source of vitamin A no other bio-assays have come to the notice of the writers. Fraps and Treichler (7) reported that samples of bur clover and sudan grass dried in vacuum contained 200 and 150 units per gram, respectively, but no data were presented on the fresh green grasses as the cows consume them.

Any loss of moisture would tend to concentrate other constituents in the grass samples and would probably indicate a higher vitamin A content than the original grass contained. Although some moisture was lost from the samples studied, the grass was still fresh and green when fed. The average moisture content of the timothy samples was 67.9 per cent and of the red top 60.5. These averages compare well with the average of 60.7 per cent for 16 analyses of red top and 62.5 per cent for 88 samples of timothy, all analyses, and 75.8 for 5 samples, before bloom, reported by Henry and Morrison (10).

The vitamin A activity of each of these four pasture plants is much higher than has been reported for most feeds or foods except some samples of fish liver oils.

Fraps, Copeland, and Treichler (8) were unable to maintain high vitamin A activity in butter when cows were fed 116,000 rat units daily. They

suggested that fresh green grass was necessary in the diet of the cow in order to keep the vitamin content of the butter high.

A dairy cow can easily consume 100 pounds of fresh green grass daily. If the grass contained 200 rat units of vitamin A activity per gram, the total intake of vitamin A activity per day would be over nine million rat units. It is not surprising that summer butter produced by cows fed unlimited quantities of fresh grass has been found to be usually higher in vitamin A activity than winter butter.

CONCLUSIONS

Results of this study indicate that the vitamin A activity of the two pasture plants, timothy (*Phleum pratense* L.) and red top (*Agrostis alba* L.), was 220 ± 13 and 308 ± 10 rat units respectively, considering a gain of 12 grams in 4 weeks as representing one unit. Statistically, this is a significant difference. Compared with white clover (242 ± 19 units) and Kentucky bluegrass (175 ± 11 units) previously reported, timothy would rank between them while red top contained a third more units than even white clover. The content of vitamin A activity in these pasture plants is higher than has been reported for any feeds or foods.

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STUDIES ON THE CHEMICAL COMPOSITION OF THE BLOOD OF DAIRY CATTLE

I. THE EFFECT OF AGE AND PHOSPHORUS INTAKE ON THE CALCIUM AND INORGANIC PHOSPHORUS CONTENT OF WHOLE BLOOD OF DAIRY HEIFERS

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The calcium and inorganic phosphorus content of the blood has been studied rather extensively in recent years in the diagnosis of phosphorus deficiency and in studies of mineral metabolism and disease.

In a study of the effect of low-mineral rations on the growth and development of dairy heifers considerable data have been obtained on the effect of age and phosphorus intake on the calcium and inorganic phosphorus content of the whole blood of growing dairy heifers.

The inorganic phosphorus is one of the constituents of the blood that is commonly thought to be influenced by the amount of phosphorus in the diet. Many of the data leading to this conclusion have been secured on small laboratory animals. However, Meigs, Blatherwick, and Cary (15), Blatherwick (4), Robinson and Huffman (20), and others have obtained results on dairy animals supporting this assertion. Theiler, Green and DuToit (24) were the first to produce an experimental "aphosphorosis" by feeding growing heifers rations low in phosphorus. They reported 5.2 mgs. of inorganic phosphorus per 100 mls. of whole blood for normal animals while the animals on the low-phosphorus rations were as low as 1.3 mgs. per 100 mls. Palmer and Eckles (18) reported low values for inorganic phosphorus in the blood of animals raised in a phosphorus deficient region. Malan, Green and DuToit (14) have shown that the blood of heifers grown on phosphorus deficient pastures is likely to be low in inorganic phosphorus. Results obtained by Henderson and Weakley (8) and Eckles and associates (5) show that when the phosphorus in the feed is lowered the inorganic phosphorus in the blood is decreased in a relatively short time. Meigs, Blatherwick, and Cary (15), Eckles and associates (5), and others have shown that the inorganic phosphorus of the blood of animals on low-phosphorus rations may be raised to normal by supplementing the ration with sodium phosphate or by feeding normal feeds high in phosphorus.

Meigs and associates (15), Palmer and Eckles (17), and Henderson and Weakley (8) have reported age as a factor affecting the inorganic phosphorus

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of the blood. Inorganic phosphorus was found to be fairly high in new-born calves and tended to increase, reaching a maximum at about six months of age. This maximum was followed by a gradual decline as the animals grew older.

Meigs and coworkers (15) reported the concentration of calcium in the plasma of cows' blood to be fairly constant. Palmer and Eckles (19) however found it subject to significant fluctuations of an undertermined cause in spite of a high coefficient of correlation on successive days.

Palmer and Eckles (18) obtained normal values for calcium when the inorganic phosphorus was much below normal. Supplementing the low phosphorus ration with calcium carbonate or sodium phosphate was without effect on the calcium content of blood plasma.

Results obtained by Anderson, Gayley, and Pratt (1) indicate no noticeable difference in the calcium of the blood of young and mature animals. However, Henderson and Weakley (8) and Huffman and associates (12) have obtained some evidence that there is a slight diminution in the calcium concentration of heifers' blood with an increase in age.

With these points in mind it was thought desirable to make a further study of the effect of age and level of phosphorus intake on the calcium and inorganic phosphorus content of the blood of dairy heifers during the period of growth and to continue this study through the periods of gestation and first lactation. It seemed probable that much information might be gained as to the minimum requirement of phosphorus for such animals. This report covers the period of growth from birth to about 25 months of age.

EXPERIMENTAL

For this study three groups of grade Holstein heifer calves were used. One group was fed a normal ration while the others were fed rations low in phosphorus.

The normal group consisted of seven calves and was fed a ration composed of timothy hay, corn meal, ground oats, wheat bran, and corn-gluten meal. The calcium and phosphorus were assumed to be present in sufficient amounts, as the ration is representative of those fed under practical farm conditions without any apparent harmful results.

The first low-phosphorus group, Group A, consisted of five animals and was fed a ration composed of timothy hay, polished rice, corn-gluten meal, and beet pulp or wheat flour. This ration was much lower in its phosphorus content than the ration fed the normal group but otherwise was very similar in its nutritive value.

The second low-phosphorus group, Group B, consisted of four animals and was fed a ration similar to that fed Group A except that wheat gluten was used in place of corn-gluten meal as the principle source of protein for the first fifteen months. After the first fifteen months the animals in Group

B were fed the same ration as were the animals in Group A. The wheat gluten supplied considerably more digestible crude protein in proportion to phosphorus than did the corn-gluten meal. As a result somewhat less phosphorus was supplied in the ration fed the calves in Group B for the first fifteen months of the experiment.

The timothy hay used would grade as U. S. No. 1 and was fed at the rate of about 1½ pounds per 100 pounds live weight per day. The remainder of the digestible crude protein and total digestible nutrients required to meet the average of the Morrison feeding standard for dairy heifers was supplied by the respective grain rations fed each group.

Feed Analysis

Analyses of all feeds for moisture, ash, protein, ether extract, crude fiber, calcium, and phosphorus were made by the usual methods on composite samples taken when new supplies of feed were purchased. Whenever possible the average analyses were converted to the digestible basis by the digestibility coefficients for American feeding stuffs as given by Henry and Morrison (10); otherwise the digestibility coefficient of some closely-related feed was used.

Feeding and Care of Animals

The calves were fed whole milk until they were about six weeks of age, after which they were gradually changed to skimmilk. The respective grain and hay rations were offered as soon as the calves would consume them. The skimmilk was gradually withdrawn from all groups and the calves transferred entirely to the grain and hay rations at the age of six months. Feed and water was offered twice daily and an accurate account was kept of the amounts consumed. Salt was incorporated in the various grain rations at the rate of 1 pound in each 100 pounds of the grain mixture.

The calves were housed in a well-lighted and ventilated experimental barn but were not exposed to direct sunlight. Each calf was kept in an individual pen of convenient size and fed separately. The animals were confined in their pens continuously except when they were removed to be weighed, measured, and photographed. Wood shavings were used as bedding.

All animals were weighed and the height at withers was measured at weekly intervals. Weights and measurements were made at about the same hour each day in order to eliminate, as much as possible, the difference in weight due to feeding and watering.

Methods of Blood Analysis

Composite samples of whole blood were collected from all animals at monthly intervals according to the method previously described (9).

Trichloroacetic acid filtrates were prepared by adding dropwise, with vigorous stirring, 12.5 mls. of whole blood to 50 mls. of 10 per cent trichloracetic acid and the mixtures allowed to stand approximately 5 minutes, after which they were filtered through a No. 40 Whitman filter paper. With this amount of whole blood and trichloroacetic acid insufficient filtrate was obtained for triplicate determinations of both calcium and inorganic phosphorus in case the usual duplicate determinations did not check and additional filtrate was desired.

Calcium was determined on 10-ml. aliquots of the trichloroacetic acid filtrate (equivalent to 2 mls. of whole blood) according to the method described by Rothwell (21).

Inorganic phosphorus was determined on 5-ml. aliquots of the trichloroacetic acid filtrate according to the method of Fiske and Subbarow (6).

Effect of Ration on Blood Composition

In Table 1 is presented the average growth in weight and height at withers and the average daily consumption of digestible crude protein, total digestible nutrients, calcium, phosphorus and phosphorus consumed per 100-pounds body weight by the animals in the various groups.

Figure 1 shows the average amount of inorganic phosphorus in the blood of the animals in the various groups along with the average daily phosphorus consumption and the amount consumed per 100-pounds body weight of the animals. The average deviation of inorganic phosphorus from the mean for the entire period was .44 mg. per 100 mls. of whole blood for the normal group, .42 mg. for the low phosphorus Group A, and .50 mg. for the low-phosphorus Group B. However, greater deviations than those given occurred in the case of the low-phosphorus groups for the period of 6 to 12 months of age.

As a general rule blood analyses were started at about the second or third months of age. However, in the case of three animals in the normal group and two animals in the low-phosphorus Group B, the blood was not analyzed until after the animals were removed from skimmilk at 6 months of age. For the normal group, results of analyses for 5 animals normally fed but not a part of this experiment were included in the average results presented up to 6 months of age. These animals were of the same breed and were confined to the experimental barn just as the animals regularly on this experiment.

In the case of the animals fed the normal-phosphorus ration there was a slight increase in the inorganic phosphorus of the blood from the second to the fourth month, after which it remained fairly constant until about the tenth month was reached. After the tenth month there was a gradual decrease as the animals grew older.

The animals fed the low-phosphorus ration in Group A showed a slight

TABLE 1

Average growth in weight and height at withers; average daily consumption of digestible protein; total digestible nutrients, calcium and phosphorus

AGE IN PERIODS	BEGINNING OF PERIOD WEIGHT AT *lbs.*	HEIGHT AT BEGINNING OF PERIOD *cms.*	DIGEST. PROTEIN *lbs.*	TOTAL DIGEST. NUTR. *lbs.*	AVE. DAILY CA. *gms.*	AVE. DAILY PHOS. *gms.*	PHOS. PER 100 POUNDS LIVE WEIGHT *gms.*
Normal Phosphorus Group							
12– 24 wks.	175	85.8	.72	3.19	9.0	10.4	4.90
24– 36 "	293	97.8	.66	5.17	7.2	13.0	4.09
36– 48 "	399	106.1	.95	7.22	10.7	19.2	4.12
48– 60 "	520	113.2	1.17	8.80	12.7	23.0	4.11
60– 72 "	638	118.7	1.34	9.82	15.0	27.3	4.00
72– 84 "	772	123.4	1.48	11.01	17.4	30.1	3.70
84– 96 "	887	127.2	1.44	11.50	19.3	30.8	3.37
96–108 "	989	130.3	1.45	11.18	20.6	31.0	3.03
Low Phosphorus Group A							
12– 24 wks.	184	85.7	.74	3.73	9.8	8.7	3.87
24– 36 "	308	98.5	.73	5.75	7.8	6.4	1.79
36– 48 "	446	108.1	1.14	7.63	10.3	8.5	1.73
48– 60 "	587	115.6	1.26	8.91	12.7	9.7	1.56
60– 72 "	688	119.7	1.36	9.50	14.1	10.6	1.48
72– 84 "	781	123.8	1.47	10.38	15.5	11.5	1.42
84– 96 "	893	126.6	1.32	10.21	16.9	11.6	1.25
96–108 "	951	129.0	1.30	10.00	16.8	11.3	1.15
Low Phosphorus Group B							
12– 24 wks.	178	85.8	.70	3.10	9.1	7.0	3.35
24– 36 "	292	96.8	.61	4.84	8.2	4.1	1.32
36– 48 "	358	104.8	.84	6.57	10.9	5.9	1.44
48– 60 "	479	111.2	.92	6.80	10.8	6.4	1.27
60– 72 "	587	116.8	1.06	8.01	13.7	8.5	1.35
72– 84 "	724	121.0	1.32	9.09	15.8	10.4	1.36
84– 96 "	833	125.0	1.41	10.52	18.0	11.8	1.35
96–108 "	966	127.7	1.48	11.40	20.4	12.9	1.27

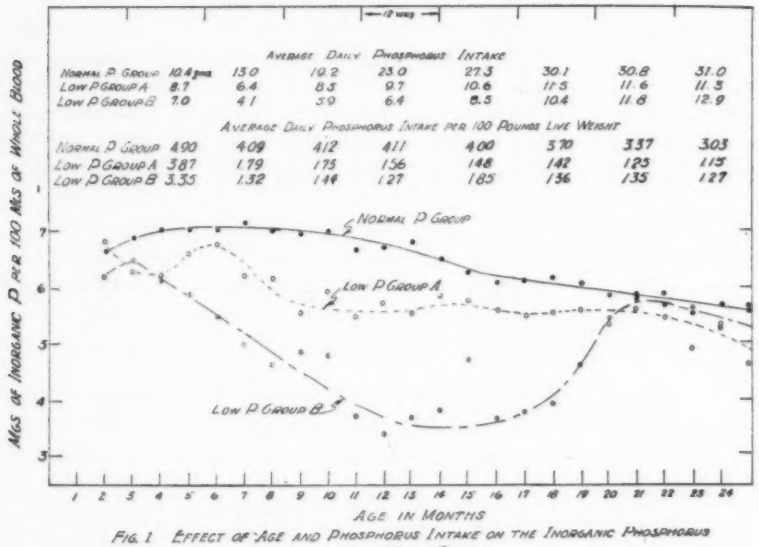
12 WKS
AVERAGE DAILY PHOSPHORUS INTAKE
NORMAL P GROUP 10.4 gms. 13.0 19.2 23.0 27.3 30.1 30.8 31.0
LOW P GROUP A 8.7 6.4 8.3 9.7 10.6 11.5 11.6 11.3
LOW P GROUP B 7.0 4.1 5.9 6.4 8.3 10.4 11.8 12.9
AVERAGE DAILY PHOSPHORUS INTAKE PER 100 POUNDS LIVE WEIGHT
NORMAL P GROUP 4.90 4.09 4.12 4.11 4.00 3.70 3.37 3.03
LOW P GROUP A 3.87 1.79 1.75 1.56 1.48 1.42 1.25 1.15
LOW P GROUP B 3.35 1.32 1.44 1.27 1.85 1.36 1.35 1.27
NORMAL P GROUP
LOW P GROUP A
LOW P GROUP B
MGS OF INORGANIC P PER 100 MLS OF WHOLE BLOOD
AGE IN MONTHS

FIG. 1 EFFECT OF AGE AND PHOSPHORUS INTAKE ON THE INORGANIC PHOSPHORUS OF WHOLE BLOOD

drop in the inorganic phosphorus of the blood for the third and fourth months, followed by a gradual rise until about the sixth month, after which there was a gradual decline until about the twentieth month was reached. During the twenty-first month the inorganic blood phosphorus rose to about the same level as that of the normal group followed by a gradual decline throughout the remainder of the period.

The animals which were fed the low-phosphorus ration in Group B and which received the least amount of phosphorus for the first 15 months of the experiment, began showing a decline in the inorganic phosphorus of the blood after the third month, which continued until the animals were about 12 months of age. At this time there was present on the average about 3.4 mgr. of inorganic phosphorus per 100-mls. of whole blood, or about half as much as was present in the blood of the animals in the normal group. The inorganic phosphorus continued at a low level until about the eighteenth month, after which there was a gradual increase until at about the twenty-first month it had risen to approximately the same level as that of the normal group. It may be noticed in Figure 1 that Group B received slightly more phosphorus than did Group A during the last two 12-week feeding periods. The inorganic phosphorus of the blood was also slightly higher. This difference was due largely to variations in the consumption of feed by the various animals.

All the heifers on the low-phorphorus ration of Group B showed very definite symptoms of phosphorus deficiency from time to time when the

inorganic phosphorus of the blood was at a low level. However, there was a period of several weeks after the inorganic phosphorus of the blood was reduced below normal, before any physical symptoms were apparent. The exact time varied with the individual animals. The most pronounced physical symptoms of phosphorus deficiency were stiffness in front and rear quarters, unsteadiness in walk, excessive nervousness when excited, and emaciated condition in general. One of these heifers began showing weakness and stiffness in front and rear quarters at about 13 months of age. She went off feed and her condition gradually grew worse until at 14½ months of age she was unable to stand without assistance. It was observed that breathing became heavy and difficult when she was excited or exerted much effort. Often times she would bawl as though in pain. She was slaughtered at the age of 15 months.

At no time did any of the animals in Group A show pronounced physical symptoms of phosphorus deficiency during the period under consideration. It may be seen in Table 1 that there was very little difference in the rate of growth of the animals in the various groups as measured by the rate of gain in body weight and height at withers. However, there was a tendency for the animals on the low phosphorus rations to show signs of loss of appetite, and to make irregular gains, but over the entire period the animals on the low-phosphorus rations made almost as much gain both in body weight and height at withers as did the animals in the normal group.

In Table 2 are presented in summarized form the average amounts of calcium and inorganic phosphorus in the whole blood of the animals in the various groups. Age did not seem to have any appreciable effect on the calcium content of whole blood, whereas there was a definite decrease with age during the second year in the case of the inorganic phosphorus. The level of phosphorus intake did not seem to have any definite effect upon the calcium in whole blood. The averages of the means for the various groups were 7.16 mgs. per 100-mls. of whole blood for the normal group, 7.06 for the low-phosphorus Group A, and 7.62 for the low-phosphorus Group B. When these values are compared there is some indication that the calcium content of the blood of the low-phosphorus Group B was slightly higher than that of the other two groups. Although that might have been the case, the difference could not be considered biologically significant, as the average deviation from the mean for the normal group was .667 mg. of calcium per 100-mls. of whole blood.

DISCUSSION

The purpose of this investigation was to study the effect of age and level of phosphorus intake on the calcium and inorganic phosphorus content of the blood in order to determine if possible the minimum phosphorus requirement for growing dairy heifers. Obviously there are a number of other

TABLE 2

The effect of age and phosphorus intake on the calcium and inorganic phosphorus content of the whole blood

(Mgs. per 100 mls. of whole blood)

AGE IN MONTHS	NORMAL P. GROUP		LOW P. GROUP A		LOW P. GROUP B	
	Ca.	Inor. P.	Ca.	Inor. P.	Ca.	Inor. P.
2		6.67		6.83		6.20
3		6.90		6.30		6.50
4	7.17	7.03	7.40	6.24	7.20	6.20
5	7.03	7.04	6.80	6.62	8.50	5.90
6	7.58	7.03	6.74	6.78	7.25	5.50
7	7.15	7.18	7.30	6.24	8.25	5.02
8	7.20	7.06	6.80	6.16	8.30	4.65
9	7.44	7.00	7.58	5.58	8.40	4.90
10	7.43	7.01	6.92	5.96	7.90	4.85
11	6.94	6.68	6.76	5.52	7.35	3.72
12	7.56	6.74	7.34	5.74	7.68	3.43
13	7.11	6.83	7.06	5.60	7.38	3.70
14	7.42	6.53	7.16	5.90	7.18	3.85
15	7.33	6.30	6.58	5.80	7.66	4.73
16	6.80	6.15	7.78	5.62	7.50	3.70
17	7.50	6.17	7.37	5.54	6.90	3.83
18	6.50	6.20	7.35	5.56	6.47	3.97
19	7.03	6.10	7.10	5.62	7.85	4.65
20	6.90	5.90	7.40	5.36	7.50	5.47
21	7.28	5.93	6.70	5.66	7.23	5.90
22	6.31	5.93	6.60	5.50	7.93	5.73
23	7.75	5.56	6.83	4.95	7.27	5.63
24	7.03	5.71	6.65	5.40	8.33	5.30

factors such as the vitamin D content of the ration, season of the year, calcium-phosphorus ratio, and possibly the source of phosphorus in the ration that might affect the level of phosphorus required and the inorganic phosphorus and calcium content of the blood. An attempt was made to control these factors in so far as was practicable.

Vitamins—No vitamins were fed other than those occurring normally in the feeds used. The heifers were confined continuously to the experimental barn, which helped to eliminate the effect of sunshine and season. The results of several investigations (11) (22) (3) show that sun-cured hay is a fairly good source of vitamin D. Work at the Michigan Station (11) indicates that sun-cured timothy hay furnishes sufficient vitamin D to protect calves kept out of sunshine from developing rickets. The heifers were fed a good grade of timothy hay at the rate of about 1½ pounds per 100-pounds of live weight. However, with more vitamin D available for calcium and phosphorus utilization the blood of the animals in the low-phosphorus groups might not have been affected to the same degree.

Calcium-Phosphorus Ratio—This experiment was not designed to study

the effect of the calcium-phosphorus ratio. However, in the case of the normal group there was always more phosphorus than calcium in the ration with an average calcium-phosphorus ratio of 1 to 1.65. In the case of the low phosphorus groups there was always more calcium than phosphorus in the ration with an average calcium-phosphorus ratio of 1 : 0.79 for the low phosphorus Group A and 1 : 0.63 for the low-phosphorus Group B. Theiler, Green, and DuToit (24) concluded that the minimum requirements for growth are higher for phosphorus than for calcium, and that a calcium-phosphorus ratio of 1 : 4.65 is not necessarily disadvantageous. Meigs and associates (16) experimented with lactating cows and concluded that phosphorus assimilation may be interfered with by an excess of calcium in the ration, and that two parts or more by weight of calcium to one of phosphorus constitutes an excess. However, Gullickson and Eckles (7) did not notice any ill effects with growing heifers fed rations in which the phosphorus content almost invariably exceeded the calcium content by nearly two to one.

Phosphorus Requirement

It may be noted in Table 1 and Figure 1 that the average daily phosphorus consumption for all groups increased as the animals increased in age and more feed was consumed, but decreased slightly in proportion to body weight. Since there was no increase in the amount of phosphorus consumed in proportion to body weight after the eighteenth month, evidently there was a decrease in the requirement for phosphorus in proportion to body weight, as indicated by the rise in the inorganic phosphorus of the blood.

Theiler (23) was of the opinion that the phosphorus requirement was reduced slightly with age. Huffman and associates (12) suggested the possibility that the phosphorus requirement for growth depends largely on the rate of growth rather than on the body weight. They also interpret the results of Hogan and Nierman as indicating that the rate of growth is a good criterion to use in determining the phosphorus requirement for growth.

In Table 3 is presented in twelve-week periods the average number of

TABLE 3

Total phosphorus consumption per unit gain in body weight and height at withers

12 WEEKS PERIOD ENDING WHEN ANIMALS WERE AT AGE OF	NORMAL P. GROUP		LOW P. GROUP A		LOW P. GROUP B	
	Grams of P. per cm. gain in height	Grams of P. per lb. gain in body wt.	Grams of P. per cm. gain in height	Grams of P. per lb. gain in body wt.	Grams of P. per cm. gain in height	Grams of P. per lb. gain in body wt.
24 wks.	73	7.4	57	5.9	54	5.2
36 "	132	10.3	56	3.9	43	5.2
48 "	224	13.3	95	5.0	78	4.1
60 "	351	16.4	199	8.1	96	4.5
72 "	488	17.1	217	9.6	170	5.2
84 "	665	22.0	345	8.6	218	8.0
96 "	835	25.4	406	16.7	367	7.5

grams of phosphorus consumed per pound gain in body weight, and also per centimeter gain in height at withers between 12 and 96 weeks of age.

It may be noted that there was a fairly consistent increase in the amount of phosphorus consumed per centimeter gain in height at withers by the animals in all groups. There was also a consistent increase in the amount of phosphorus consumed per pound gain in body weight by the animals in the normal group. However, in the case of the animals on low-phosphorus rations the rate of increase of phosphorus consumption per pound gain was much less and very irregular. The relative change in the amounts of phosphorus consumed per centimeter gain in height at withers from 12 to 96 weeks of age was much more than the relative change in the amount of phosphorus consumed per pound gain in body weight. From a study of the changes in the inorganic phosphorus content of the blood, the data indicate that the phosphorus requirement for growth depends to a much greater degree upon the rate of skeletal growth as measured by the rate of gain in height at withers than upon the rate of gain in body weight of the animal. Even though there was a decrease in the amount of phosphorus consumed per 100-pounds body weight as the animals increased in age, nevertheless the amount of phosphorus consumed by the animals in the low-phosphorus groups during the latter part of the experiment was evidently about sufficient to meet their requirements for phosphorus. This resulted in an increase of the inorganic phosphorus in the blood to approximately the same level as that of the normal group.

While there is no definite knowledge as to the optimum amount of phosphorus required by growing dairy animals, several investigators have stated what they believed to be the requirement for such animals, while others have calculated the amounts retained by such animals.

Kellner (13) computed the phosphorus retention of growing calves as 19 grams of P_2O_5 equivalent to 8.3 grams of phosphorus per day for the first year. He was of the opinion that the ration should contain about two or three times this amount. Armsby (2) computed from Lawes and Gilbert's analyses of the ash of the entire body of farm animals that during the first year cattle gained a total of 2972 grams of phosphorus. From this Armsby calculated an average daily retention of 8.14 grams equivalent to 1.7 grams of phosphorus per 100 pounds body weight per animal. This is in close agreement with the results obtained by Kellner (13).

In a study of the mineral requirements of cattle, Theiler, Green, and DuToit (23) found that a ration supplying 2.32 grams of phosphorus and 4.99 grams of calcium per day was deficient. Animals 12 to 18 months of age withstood this level of mineral intake from three to six months after which they showed symptoms of mineral deficiency. When the basal ration was supplemented with 25 grams of bone meal daily, the phosphorus intake was increased to 5 grams per day, and fair growth resulted. When the

bone meal was increased to 100 grams and the phosphorus increased to 13 grams daily, good growth and reproduction resulted. The addition of 2 pounds of wheat bran per day to the basal ration increased the daily phosphorus intake to 11 grams, which resulted in fair gain in body weight. Theiler and associates (25) stated that in any given ration it is the percentage of phosphorus in the ration in relation to the total feeding value which is of importance in causing "osteophagia" or bone chewing of cattle. They found that for cattle of approximately 1000 pounds live weight, a daily consumption of about 11.79 grams of phosphorus per 1000 pounds body weight was somewhere near the point at which osteophagia could develop or disappear. Huffman and associates (12) obtained data which indicated that 10 grams of phosphorus per day furnishes sufficient phosphorus to first calving, especially when calves are fed whole milk for two months, five pounds of sun-cured alfalfa hay per day, and turned into sunshine daily. An intake of 10 to 12 grams per day seemed to be about sufficient to support maintenance, normal growth, and gestation from 18 months of age to first calving.

Since the composition of the blood is a good index as to the severity of phosphorus deficiency, if we assume that the lowering of the inorganic phosphorus in the blood is in direct proportion to the deficiency of phosphorus intake in the feed, the minimum phosphorus consumption required to maintain a normal supply of inorganic phosphorus in the blood under the conditions of this experiment may be calculated. If it is considered that, when the concentration of inorganic phosphorus in the blood is at a normal level, the consumption is sufficient to meet the requirement of the animal, the calculated values would be equal to the minimum requirement for phosphorus.

The calculated values for the average daily requirement under the conditions of this experiment in 12-week periods from 24 to 108 weeks of age are presented in Figure 2. The calculated values for the average daily requirement per 100-pounds body weight were obtained by dividing the amount required per day by the average body weight of all the animals on experiment for the corresponding period. The regression equations were obtained by the method of "least squares," using the calculated values for the average daily requirement, and the average daily requirement per 100-pounds body weight. In the equations "y" represents the requirement in grams per day and "X" the age of the animals in months. The regression lines were plotted according to the regression equation for the average daily phosphorus requirements and the average daily phosphorus requirements per 100-pounds body weight.

It is to be admitted that the severity of phosphorus deficiency as indicated by the lowering of inorganic phosphorus in the blood may not be directly proportional to the deficiency of the phosphorus intake in the feed,

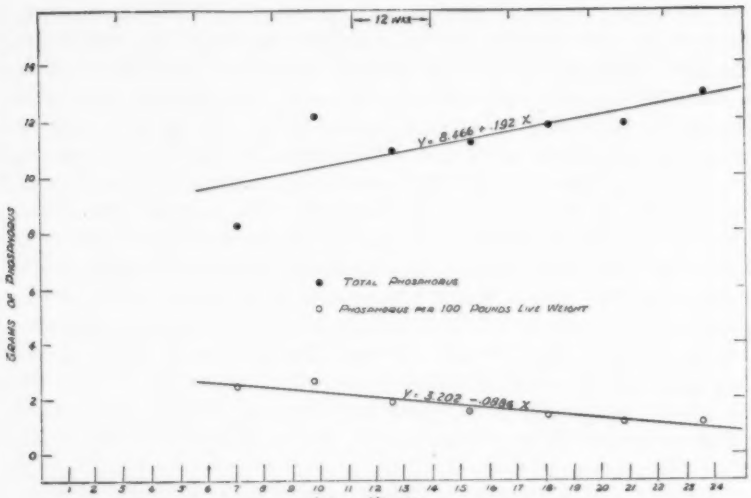

FIG. 2: CALCULATED AVERAGE DAILY MINIMUM PHOSPHORUS REQUIREMENT FOR GROWING DAIRY HEIFERS

especially during the first 48 weeks of age, when the animals were being depleted of their phosphorus reserve. It is commonly known that such factors as age, ration, vitamins, and level of phosphorus intake effect the efficiency of phosphorus absorption and storage. From balance studies it has been demonstrated that phosphorus is used more efficiently at lower levels of intake than at higher levels. There are also differences in the rate at which the inorganic phosphorus in the blood is decreased with lowered phosphorus consumption. The rate at which the composition of the blood is lowered is roughly in proportion to the level of phosphorus intake as may be seen in Figure 1, although the individuality of the animal is an important factor.

For an approximation it is interesting to note that the calculated requirements according to the regression equations are of the same order of magnitude as the values for minimum requirements of growing dairy animals obtained by other investigators. The calculated values thus obtained are slightly higher than an average of 10 grams per day as obtained by Huffman and associates (12) for heifers to first calving. The animals in this experiment were fed timothy hay and confined to the barn continuously. Probably with more vitamin D and sunshine less phosphorus would be required to maintain the inorganic phosphorus of the blood at the normal level.

The requirement for phosphorus was not calculated for the period between 3 and 6 months of age. There was a difference in the trend shown

by the inorganic phosphorus in the blood of the animals in the low-phosphorus Groups A and B during this period, although the average composition of the blood of the two groups was practically the same. When the average composition of the blood of both low-phosphorus groups was compared with that of the normal group there was a difference of .82 mgs. per 100 mls., indicating a slight deficiency of phosphorus for the animals in the low-phosphorus groups. The average daily phosphorus consumption of the animals in the low-phosphorus groups was 7.9 grams and for the animals in the normal group, 10.4 grams. The requirement for phosphorus during the period evidently falls between these two values. If the regression line be extrapolated back to include this period or the requirement calculated according to the regression equations, the calculated average daily requirement would be 9.3 grams for the period of 3 to 6 months of age. Huffman and associates (12) found that 10.3 grams of phosphorus per day appeared to furnish sufficient phosphorus for heifers from 3 to 6 months of age under the conditions of their experiment.

TABLE 4

The minimum phosphorus requirements for normal growth of dairy heifers

AGE IN MONTHS	*ESTIMATED P. REQUIREMENTS (GRAMS)	CALC. P. REQUIREMENT (GRAMS)	CALC. P. REQUIREMENT PER 100 LBS. BODY WT. (GRAMS)
1	6.94	8.7	3.11
2	8.19	8.9	3.02
3	7.74	9.0	2.94
4	11.37	9.2	2.85
5	12.58	9.4	2.76
6	12.08	9.6	2.67
7	10.60	9.8	2.58
8	10.10	10.0	2.49
9	11.39	10.2	2.40
10	10.44	10.4	2.32
11	9.24	10.6	2.23
12	9.68	10.8	2.14
13	7.20	11.0	2.05
14	8.03	11.2	1.96
15	7.47	11.4	1.87
16	11.05	11.5	1.78
17	8.05	11.7	1.70
18	10.01	11.9	1.61
19		12.1	1.52
20		12.3	1.43
21		12.5	1.35
22		12.7	1.26
23		12.9	1.17
24		13.1	1.09

* Estimated by Huffman and Associates (12).

Huffman and coworkers (12) have estimated the phosphorus requirements for normal growth to 18 months of age, based on Eckles normals and Wilson's requirements for maintenance and growth.

In Table 4 is presented the phosphorus requirements for normal growth as estimated by Huffman and associates (12) along with the calculated values from this experiment according to the regression equations. For comparison values have been calculated for the ages from 1 to 6 months. However, the experimental data used in developing the equations did not cover this period.

SUMMARY AND CONCLUSIONS

Three groups of growing dairy heifers were fed in such a way that all received approximately the same amount of digestible crude protein and total digestible nutrients in proportion to body weight. One of the groups was fed a normal ration while the others were fed rations low in phosphorus. Calcium and inorganic phosphorus was determined on composite samples of whole blood at monthly intervals throughout the experiment.

Under the conditions of the experiment as outlined the following conclusions were reached:

1. The inorganic phosphorus in the blood of animals fed a normal phosphorus ration shows a slight increase from the second to the fourth month, after which it remains fairly constant until the tenth month, when there is a gradual decline as the animals grow older.

2. The concentration of inorganic phosphorus in the blood is an important index of the severity of phosphorus deficiency in the ration. Low-phosphorus rations cause an immediate lowering of the inorganic phosphorus in the blood. The rate of lowering is roughly proportional to the severity of phosphorus deficiency in the ration. There is always a lowering of the inorganic phosphorus in the blood for a period of several weeks before any pronounced physical symptoms of a deficiency are apparent.

3. Anorexia, or loss of appetite, stiffness in front and rear quarters, and general emaciated condition are physical symptoms which usually follow a lowering of the inorganic phosphorus in the blood when animals are on phosphorus deficient rations.

4. The phosphorus requirement for growing dairy animals is not directly proportional to gain in body weight, but depends to a considerable degree upon the rate of skeletal growth as measured by the rate of gain in height at withers. There is a decrease in the requirement for growth in proportion to body weight with increase in age as the animals approach maturity.

5. An average daily phosphorus intake of approximately 25 grams (equivalent to about 3.8 grams per 100-pounds body weight) was sufficient to maintain what appeared to be a normal supply of inorganic phosphorus in the blood up to 25 months of age. An average daily phosphorus intake

of 8 grams (equivalent to 1.3 grams per 100-pounds body weight) was not sufficient to maintain a normal supply of inorganic phosphorus in the blood of growing dairy animals from 6 to 25 months of age.

6. Age and level of phosphorus intake were without effect upon the calcium content of whole blood of growing dairy heifers.

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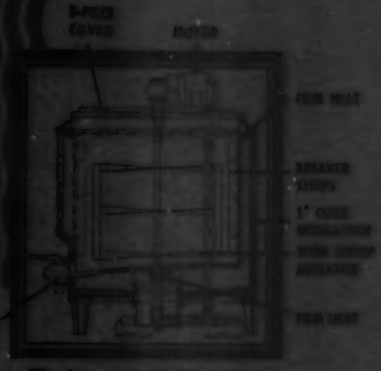

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